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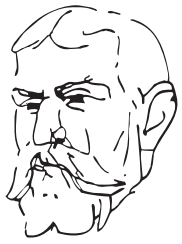

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ORIGINAL ARTICLE

# EPISIOTOMY INFECTIONS IN THE PUERPERIUM AND ANTIMICROBIAL RESISTANCE OF RESPONSIBLE PATHOGENS IN UKRAINE

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## ABSTRACT

**The aim:** To obtain the first estimates of the current prevalence rate of episiotomy infections in the puerperium and antimicrobial resistance of responsible pathogens in Ukraine.

**Materials and methods:** We performed a retrospective multicenter cohort study was based on surveillance data. The study population consisted of all women who had a vaginal delivery in 7 Regional Women's Hospitals of Ukraine. Definitions of episiotomy infections were used from the Centers for Disease Control and Prevention's National Healthcare Safety Network (CDC/NHSN).

**Results:** Total 35.6% women after vaginal delivery had episiotomy done. The prevalence rate of episiotomy infections was 17.7%. The predominant pathogens were: *Escherichia coli* (49.2%), *Enterobacter* spp. (11.1%), *Streptococcus* spp. (9.1%), *Enterococcus faecalis* (6.5%), *Klebsiella* spp. (8.1%), followed by *Pseudomonas aeruginosa* (4.7%), *Staphylococcus aureus* (4.2%), *Proteus* spp.(2.9%) and *Staphylococcus epidermidis* (2.8%). The overall proportion of methicillin-resistance was observed in 17.3% of *Staphylococcus aureus* (MRSA). Vancomycin resistance was observed in 6.8% of isolated enterococci. Carbapenem resistance was identified in 8% of *P.aeruginosa* isolates. Resistance to third-generation cephalosporins was observed in 15.2% *Klebsiella* spp. and *E.coli* 16.4% isolates. The overall proportion of extended spectrum beta-lactamases (ESBL) production among Enterobacteriaceae was 26.4%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (31.4%, vs 12.5%).

**Conclusions:** Episiotomy infections in the puerperium are common in Ukraine and most of these infections caused by antibiotic-resistant bacteria. Optimizing the management and empirical antimicrobial therapy may reduce the burden of episiotomy infections, but prevention is the key element.

**KEY WORDS:** Episiotomy, vaginal delivery, antimicrobial resistance, pathogens

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## INTRODUCTION

Episiotomy infections in the puerperal period can pose a significant risk of morbidity to women. These infections interrupt postpartum restoration; increase the potential for readmission to a health care facility. In addition, unrecognized or improperly treated genital tract infection could extend to other sites and increase the risk of severe complications or sepsis [1]. Despite the widespread application of standard aseptic techniques during vaginal birth, post-pregnancy infections remain a significant source of maternal morbidity.

In literature, episiotomy infections are reported as being rare at a rate [2]. However, the true incidence of bacterial infections in the puerperium is not fully understood as outpatient surveillance data are lacking. The most study have documented endometritis, mastitis, urinary tract infections (UTIs), and episiotomy infections at higher rates than reported by hospital surveillance systems [3-6].

Current guidelines for the treatment of infections recommend the immediate prescription of antimicrobial medicines as soon as the infection is diagnosed. Broad spectrum antimicrobials should be prescribed even before the culture results are known in order to cure the most probable infection agents [7]. Targeted antibacterial treatment should be provided following the identification of an etiological agent and resistance status. However, the results of numerous investigations prove that the prescription of an inadequate starting therapy raises the mortality rate among patients with severe infections by 1.5 – 3 times [8, 9]. In addition, inadequate therapy extends the duration of hospitalization and provokes a need for additional courses of antimicrobial therapy that makes treatment more expensive. Literature data on the etiology and resistance of pathogens caused postpartum infections varies considerably [3, 4, 6].

The epidemiology of postpartum infections in Ukraine and associated treatment outcomes are not well studied. National network for the surveillance of antimicrobial resistance is not in Ukraine [10, 11]. Previous reports of postpartum infection in Ukraine were limited [3,4].

## THE AIM

The aim of this study was to obtain the first estimates of the current prevalence rate of episiotomy infections in the puerperium and antimicrobial resistance of responsible pathogens in Ukraine

## MATERIALS AND METHODS

### STUDY DESIGN AND SETTING

A retrospective cohort study was based on surveillance data for episiotomy infections in the puerperium and included all women's who had undergone vaginal delivery at the 7 (tertiary) Regional Women's Hospitals of Ukraine. The study was conducted between January 1st, 2017 and December 31st, 2019. These hospitals provide care to individuals living within its catchment area (total 2 132 450 women's) and regularly take referrals from other (secondary) hospitals. We have included women's hospitals that are similar in terms of medical equipment, personnel, and laboratory facilities. The hospitals had 525 beds. All participating hospitals were required to have at least one full-time infection-control professional, a clinical microbiology laboratory with the capacity to process cultures.

### DEFINITION

In our study the CDC/NHSN (Centers for Disease Control and Prevention/National Healthcare Safety Network, Atlanta, Georgia, USA) definition [12] of episiotomy infections was used. Episiotomy infections must meet at least 1 of the following criteria: (1) postvaginal delivery patient has purulent drain age from the episiotomy or (2) postvaginal delivery patient has an episiotomy abscess. Episiotomy is not considered an operative procedure in NHSN.

### DATA COLLECTION

The inclusion criterion was having had an assisted vaginal delivery at Regional Women's Hospitals. Women who had been submitted to episiotomy were considered cases, while those who had not undergone episiotomy were admitted as controls. In this study, we analyzed the inpatient data and ambulatory medical records to identify episiotomy infections and describe the epidemiology of these infections. A standard data collection form was created to extract demographic and clinical data, microbiology (isolated pathogens and their antibiograms) and outcome information from inpatient data and ambulatory medical records. We collected the data using structured questionnaires adapted from based on the definition used by CDC/NHSN on episiotomy infections. The follow-up of each patient was in hospitalization period and continued during 10 days after discharge.

### MICROBIOLOGICAL SAMPLING AND SUSCEPTIBILITY TESTING

Samples were taken from women which clinical episiotomy infections. Microbial isolates were identified using standard microbiological techniques, including automated microbiology testing (Vitek-2; bioMe´rieux, Marcy l'Etoile, France), and antibiotic susceptibility testing was performed by using the disk diffusion method (Kirby – Bauer antibiotic testing) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Strains showing inhibition zone diameters in the intermediate range were considered resistant.

### ETHICS

The Shupyk National Medical Academy of Postgraduate Education (Kyiv, Ukraine) ethics committee approved this study. All the women voluntarily agreed to participate in the study and signed an informed consent form. All patient data were anonymised prior to the analysis. Ethical considerations including privacy of personal data were considered during all steps of the research.

### STATISTICAL ANALYSIS

The incidence of episiotomy infections was reported as the percentage of the total number of vaginal delivery patients who had an episiotomy procedure. The analysis of statistical data was performed using Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. Statistical significance was defined as $P<0.05$.

## RESULTS

### EPISIOTOMY INFECTIONS AND PATIENT CHARACTERISTICS

During the study period a total of 25854 participants had vaginal deliveries were included in the analysis. The prevalence of episiotomy procedures was 35.6 % (9,213/25854). In this study recruited both primiparous and women of higher parity. The rate of episiotomy procedure was determined as 73.1% in primipara women and as 24.7% in multipara women. The incidence of episiotomy infections was 17.7% [95% CI 16.8%, 18.7%]. Of the total cases episiotomy infections, 74.8% (6,847/1,628) were detected after hospital discharge. For these post-discharge infections, 68.7% of patients did not return to the hospital where they delivered for evaluation or treatment. The participants were aged between 16 and 32 years, with a mean age of 20.9 years and a standard deviation of (SD) 2.9. The majority of the study participants with episiotomy infections 1469 (18.6%) were married and had attained secondary school education 1470 (41.3%). More than half of the participants were housewives 1295 (20.8%). Characteristics of patients with episiotomy infections are shown in Table I.

### ANTIBIOTIC PROPHYLAXIS

Of 9213 participants who underwent chart review, 8278 (89.9%) were prescribed combination ceftriaxone and metronidazole postpartum, though there was little documentation of antibiotic receipt. Ceftriaxone and metronidazole was also prescribed for 1234/1628 (75.8%)

**Table I.** Characteristics of patients with episiotomy infections in Ukraine

| Characteristics | Total cohort (n=9,213) | Episiotomy infections (n=1,628) | | Incidence of episiotomy infections [95% CI[a]] |
|---|---|---|---|---|
| | | n | % | |
| Age (years, mean, SD[b]) | 20.9 | 17.1 | | |
| Age category[c] | | | | |
| ≤16 | 148 | 18 | 12.2 | 9.5 – 14.9 |
| 17- 21 | 4,894 | 844 | 17.2 | 16.7 – 17.7 |
| 22 - 26 | 3,168 | 488 | 15.4 | 14.8 – 16.0 |
| 27-31 | 760 | 211 | 27.8 | 25.6 – 29.4 |
| ≥ 32 | 243 | 67 | 27.6 | 24.7 – 30.5 |
| Gestational Age (weeks) | | | | |
| <37 | 388 | 37 | 9.5 | 7.1 – 11.9 |
| ≥37 | 8825 | 1,591 | 18.0 | 17.0 – 19.0 |
| Marital status | | | | |
| Married | 7,879 | 1,469 | 18.6 | 17.6 – 19.6 |
| Single | 1,186 | 146 | 12.3 | 11.4 – 13.3 |
| Separated/Divorced | 148 | 11 | 7.4 | 5.3 – 9.9 |
| Education level | | | | |
| Secondary | 3,563 | 1470 | 41.3 | 40.0 – 42.6 |
| College/University | 987 | 158 | 16.0 | 14.8 – 17.2 |
| Occupation | | | | |
| Housewife | 6,214 | 1,295 | 20.8 | 20.3 – 21.3 |
| Employed | 2,555 | 316 | 12.4 | 11.8 – 13.1 |
| Business | 444 | 17 | 3.8 | 2.9 – 4.7 |
| Total | 9,213 | 1,628 | 17.7 | 16.8 – 18.7 |

Note: a – Confidence interval. b – Standard deviation.

**Table II.** Distribution of pathogens, isolated from episiotomy wound secretion samples.

| Microorganism | Frequency (n=2893) | Percentage (%) |
|---|---|---|
| *Gram-positive cocci* | 653 | 22.6 |
| *S. aureus* | 122 | 4.2 |
| *S. epidermidis* | 82 | 2.8 |
| *Streptococcus spp.* | 261 | 9.1 |
| *E. faecalis* | 188 | 6.5 |
| *Gram-negative bacilli* | 2219 | 76.7 |
| *E. coli* | 1423 | 49.2 |
| *Enterobacter spp.* | 322 | 11.1 |
| *Klebsiella spp.* | 255 | 8.1 |
| *Proteus spp.* | 83 | 2.9 |
| *P. aeruginosa* | 136 | 4.7 |
| *Fungi* | 21 | 0.7 |
| *Candida albicans* | 18 | 0.6 |
| Other | 3 | 0.1 |

participants meeting criteria for episiotomy infections. Another 227/1628 (13.9%) had no antibiotic prescription, and 167/1628 (10.3%) were prescribed alternative antibiotic regimens. Overall, 89,9% participants had a chart-documented prescription for β-lactam antibiotic prophylaxis, including 75,8% of participants with episiotomy infection.

### PATHOGENS CAUSING OF EPISIOTOMY INFECTIONS

A total of 2893 different bacterial strains were isolated from 1628 women's with episiotomy infections. In the present study, 6.2% samples did not show any microbial growth. It is possible that the media/conditions used in the study were not favourable for the growth of microorganisms present in these samples. Aerobic gram-negative bacilli make up 76.7% and 22.6% gram-positive cocci from of all isolates. The predominant pathogens were: *Escherichia coli* (49.2%), *Enterobacter* spp. (11.1%), *Streptococcus* spp. (9.1%), *Enterococcus faecalis* (6.5%), *Klebsiella* spp. (8.1%), followed by *Pseudomonas aeruginosa* (4.7%), *Staphylococcus aureus* (4.2%), *Proteus* spp. (2.9%) and *Staphylococcus epidermidis* (2.8%). The distribution of the microorganisms causing of episiotony infection are shown in Table II.

### ANTIMICROBIAL RESISTANCE OF RESPONSIBLE PATHOGENS

Antimicrobial susceptibility tests were performed on a total of 653 isolates of Gram-positive cocci and 2219 gram-negative organisms. The antimicrobials used in antimicrobial susceptibility testing included those commonly used as therapeutic agents. Varying degrees of resistance to most antimicrobials tested were found. The antibiotic susceptibility profiles of isolates from women's with episiotomy infections are presented in Table III and Table IV.

The overall proportion of methicillin-resistance was observed in 17.3% of *Staphylococcus aureus* (MRSA). Vancomycin resistance was observed in 6.8% of isolated enterococci (VRE). Carbapenem resistance was identified in 8% of *P.aeruginosa* isolates. Resistance to third-generation cephalosporins was observed in 15.2% *Klebsiella* spp. and *E.coli* 16.4% isolates. The overall proportion of extended spectrum beta-lactamases (ESBL) production among Enterobacteriaceae was 26.4%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (31.4%, vs 12.5%).

## DISCUSSION

This is the first study were to obtain of the current prevalence rate of episiotomy infections in the puerperium and antimicrobial resistance of responsible pathogens in Ukraine.

Episiotomy is one of the most frequent surgical interventions performed in obstetrics. One reason for episiotomy is perineal protection from severe lacerations. Prevalence of episiotomy varies significantly between countries. The primiparity has been seen in many studies as an important risk factor for episiotomy. The rate of episiotomy varies between 9.7% and 100% in both primipara and multipara women [13-18]. In our study, the prevalence of episiotomy was 35.6%.

Episiotomy is obstetric procedure, due to its special anatomy position, carelessness treatment can lead to incision infection, which is mainly related to its susceptibility to vaginal, intestinal and urethral microbial flora infection. Currently routine episiotomy is no longer performed, however infections still occur and are often seen post-vaginal or operative vaginal delivery. According to the literature data, the episiotomy infection rate was from 0.3% to 10.42% [19]. In our study prevalence of episiotomy infection rate was 17.7%.

According to the literature data, pathogens of episiotomy infections are gram-negative bacilli and gram-positive cocci. Previous studies have shown that the episiotomy infection pathogen bacteria of infection were mainly gram negative bacteria [20]. In our study the pathogen bacteria of infection were also mainly gram negative bacteria. The predominant pathogens were: *Escherichia coli* (49.2%), *Enterobacter* spp. (11.1%), *Streptococcus* spp. (9.1%), *Enterococcus faecalis* (6.5%), *Klebsiella* spp. (8.1%), followed by *Pseudomonas aeruginosa* (4.7%), *Staphylococcus aureus* (4.2%), *Proteus* spp.(2.9%) and *Staphylococcus epidermidis* (2.8%).

In this study the overall proportion of MRSA was observed in 17.3% and of VRE in 6.8%. Carbapenem resistance was identified in 8% of *P.aeruginosa* isolates. Resistance to third-generation cephalosporins was observed in 15.2% *Klebsiella* spp. and *E.coli* 16.4% isolates. The overall proportion of ESBL production among Enterobacteriaceae was 26.4%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae*.

Bacterial infections occurring during labour, childbirth, and the puerperium may be associated with considerable maternal morbidity. Antibiotic prophylaxis might reduce wound infection incidence after an episiotomy, particularly in situations associated with a higher risk of postpartum perineal infection [21]. However, available evidence is unclear concerning the role of prophylactic antibiotics in preventing infections after an episiotomy. In our study, it was observed that prophylactic antibiotics had little effect on infection cases in episiotomy following normal vaginal delivery, but further studies is needed come to a more definitive conclusion.

Antibiotic prophylaxis is one of the methods to reduce the risk of post-partum infections. The purpose of antibiotic prophylaxis is to reduce the colonisation pressure of microorganisms introduced at the time of operation to a level that the patient's immune system is able to overcome [22]. Routine antibiotic prophylaxis is not recommended after an episiotomy or repair of an obstetric laceration [22]. However, infection increases the risk of perineal repair breakdown, particularly for higher-order (third- or fourth-degree) lacerations [23]. General infection control measures, such as hand hygiene, aseptic surgical techniques, disinfection of the surgical site, and sterilisation of instruments can help minimise the risk of episiotomy infection [24]. Because puerperal genital tract infection usually begins after discharge, detailed education for women will encourage preventative health care, prompt recognition, and treatment.

Our results indicate that episiotomy infections requiring medical attention are common and that most infections occur after hospital discharge, so that use of routine inpatient

**Table III.** Antibiotic susceptibility of gram-positive bacteria

| Antibiotic | *S. aureus* (n = 122) | | *S.epidermidis* (n = 82) | | *Streptococcus spp.* (n = 261) | | *E. faecalis* (n = 188) | |
|---|---|---|---|---|---|---|---|---|
| | S | R | S | R | S | R | S | R |
| Benzylpenicillin | 28.3 | 71.7 | 30.7 | 69.3 | 35.2 | 64.8 | 72.9 | 27.1 |
| Ampicillin | 81.7 | 18.3 | 89.2 | 10.8 | 48.3 | 51.7 | 88.1 | 11.9 |
| Oxacillin | 82.7 | 17.3 | 79.6 | 20.4 | NT | NT | NT | NT |
| Cefuroxime | 88.9 | 17.1 | 78.6 | 21.4 | 94.1 | 5.9 | 38.8 | 61.2 |
| Cefotaxime | 82.5 | 17.5 | 89.8 | 10.2 | 85.2 | 14.8 | NT | NT |
| Ceftriaxone | 68.8 | 31.2 | 81.5 | 18.5 | 86.2 | 13.8 | NT | NT |
| Imipenem | NT | NT | NT | NT | NT | NT | 100.0 | 0 |
| Gentamycin | 87.4 | 12.6 | 91.5 | 8.5 | 91.2 | 8.8 | 81.1 | 18.8 |
| Tobramycin | 92.4 | 7.6 | 100.0 | 0 | 95.9 | 4.1 | 76.8 | 23.2 |
| Ciprofloxacin | 78.3 | 21.7 | NT | NT | NT | NT | 91.1 | 8.9 |
| Levofloxacin | 83.7 | 16.3 | 74.2 | 25.8 | 87.2 | 12.8 | 92.8 | 7.2 |
| Erythromycin | 34.1 | 65.9 | 28.4 | 71.6 | 31.2 | 68.8 | 15.1 | 84.9 |
| Clindamycin | 83.5 | 16.5 | 78.8 | 21.2 | 89.8 | 16.2 | 11.9 | 88.1 |
| Linezolid | 100.0 | 0 | 100.0 | 0 | 99.8 | 0.2 | 100.0 | 0 |
| Vancomycin | 100.0 | 0 | 100.0 | 0 | NT | NT | 93.2 | 6.8 |
| Tigecycline | 100.0 | 0 | 94.1 | 5.9 | 69.4 | 30.6 | 100.0 | 0 |
| Fusidic acid | 100.0 | 0 | 58.6 | 41.4 | NT | NT | NT | NT |

R, resistant isolates (%); S, susceptible isolates (%); NT, no tested;

**Table IV.** Antibiotic susceptibility of gram- negative bacteria

| Antibiotic | *E. coli* (n=1423) | | *Enterobacter spp.* (n=322) | | *Klebsiella spp.* (n=255) | | *Proteus spp.* (n=83) | | *P. aeruginosa* (n=136) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S | R | S | R | S | R | S | R | S | R |
| Amoxicillin | 65.2 | 34.8 | NT | NT | NT | NT | 70.4 | 29.6 | NT | NT |
| AMC | 78.1 | 21.9 | 39.8 | 60.2 | 85.2 | 14.8 | 84.3 | 15.7 | NT | NT |
| Ticarcillin | 69.9 | 30.1 | 92.7 | 7.3 | NT | NT | 86.5 | 13.5 | 81.9 | 18.1 |
| TZP | 96.3 | 3.7 | 96.5 | 3.5 | 100.0 | 0 | 100 | 0 | 77.2 | 22.8 |
| Cefuroxime | 63.8 | 36.2 | 77.4 | 22.6 | 87.6 | 12.4 | NT | NT | NT | NT |
| Cefotaxime | 87.1 | 12.9 | 96.1 | 3.9 | 88.3 | 11.7 | 98.8 | 1.2 | NT | NT |
| Ceftriaxone | 72.2 | 27.8 | 65.9 | 34.1 | 73.9 | 26.1 | NT | NT | NT | NT |
| Ceftazidime | 91.4 | 8.6 | 96.2 | 3.8 | 92.1 | 7.9 | 94.5 | 5.5 | 87.8 | 12.2 |
| Cefepime | 93.3 | 6.7 | 100.0 | 0 | 77.6 | 22.4 | 96.7 | 3.3 | 51.2 | 48.8 |
| Imipenem | 87.1 | 12.9 | 100.0 | 0 | 91.3 | 8.7 | 98.3 | 1.7 | 84.6 | 15.4 |
| Meropenem | NT | NT | NT | NT | NT | NT | NT | NT | 91.3 | 8.7 |
| Ertapenem | 100.0 | 0 | 100.0 | 0 | 100 | 0 | 100 | 0 | 100.0 | 0 |
| Gentamycin | 94.5 | 5.5 | 91,2 | 8.8 | 91.2 | 8.8 | 98.8 | 1.2 | 63.8 | 36.2 |
| Amikacin | 89.4 | 10.6 | 92.7 | 7.3 | 82.6 | 17.4 | 100.0 | 0 | 84.7 | 15.3 |
| Ciprofloxacin | 87.2 | 12.8 | 98.6 | 1.4 | 95.1 | 4.9 | 75.1 | 24.9 | 81.2 | 18.8 |
| Levofloxacin | 67.3 | 32.7 | 78.7 | 21.3 | 92.7 | 7.3 | NT | NT | NT | NT |
| Cefoperazone | NT | NT | NT | NT | NT | NT | NT | NT | 66.2 | 33.8 |

R, resistant isolates (%); S, susceptible isolates (%); NT, no tested; AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam.

surveillance methods alone will lead to underestimation of episiotomy infections rates. Use of information collected from hospital and ambulatory records allows efficient identification of women who are very likely to have episiotomy infections.

Information resulting from more complete surveillance could be used to identify settings with unusually high or low infection rates to identify practices associated with lower infection rates. This information could then be used to focus, motivate, and assess the effectiveness of practice changes aimed at improving infection rates in all settings. Additional research is needed to evaluate the generalizability of this surveillance methodology, and to assess resource utilization associated with these infections. Strategic planning and implementation of postpartum infections surveillance is required.

## CONCLUSIONS

Episiotomy infections in the puerperium are common in Ukraine and most of these infections caused by antibiotic-resistant bacteria. Postpartum episiotomy infection often begins and is diagnosed after discharge. Detailed education for women is needed, which can preventative health care, prompt recognition, and treatment. Antibiotics for treatment in episiotomy infection following vaginal delivery should be used in light of the local antimicrobial resistance data. Optimizing the management and empirical antimicrobial therapy may reduce the burden of episiotomy infections, but prevention is the key element. Knowledge about local data of resistance may contribute to limiting resistance and may have a significant role in designing effective antimicrobial stewardship policies.

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ORIGINAL ARTICLE

# THE IMPACT OF THE PANDEMIC LOCKDOWN ON AIR POLLUTION, HEALTH AND ECONOMIC GROWTH: SYSTEM DYNAMICS ANALYSIS

10.36740/WLek202011102

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## ABSTRACT

**The aim** of the paper is checking the hypothesis on the linking between consequences of pandemic lockdown and air pollution, public health, and economic growth.

**Materials and methods:** for prediction and modelling of the pandemic lockdown's impact on the air pollution, health, and economic growth with the system dynamics analysis and software Vensim; for the analysis, the authors used the methods as follows: bibliometric analysis with Scopus Tools Analysis and software VOSviewer.

**Results:** The findings confirmed that the current rate of infected from growing disease was 11%. If quarantine continues the rate of infected from the growing disease will be 15%. If the quarantine cancels the rate of infected from the growing disease will be 5%, and the declining of GDP increment will be higher, than in scenario with quarantine.

**Conclusions:** The findings confirmed the hypothesis that lockdown has the negative impact on the economic, social, and ecological growth of the country. At the same time, in the case, if the government cancel the quarantine, the declining of GDP increment will be higher, and the rate of infected from the growing disease will be the highest – 15%. In this case, the government should provide the quarantine regime and strengthen the control of the compliance.

**KEY WORDS:** economic growth, health economics, pandemic, public health

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## INTRODUCTION

The pandemic lockdowns have an impact in all spheres from public health to economic and ecological development of the country. The scientists proved that lockdown is the most effective way to stop the extending and penetration of virus among society. Form the other side lockdown leads to the collisions between the economic, the social, and the ecological spheres. The findings of a retrospective analysis of consequences from pandemic confirmed the existence of the direct (public health) and indirect effects (economic, social, and ecological development of the country). Thus, considering the findings in the paper [1] in the pandemic periods (on the United Kingdom examples 1270-2019 years) the death rate increased, and GDP per capita decreased which limit the financing into the healthcare sector in the country. Besides, the scientists concluded that the highest level of unemployment was during the lockdown under the Great Pandemic (1870–1875) and the Russian flu [1].

The new wave of coronavirus disturbs the whole world and has provoked the vulnerable and imbalance the social and economic development. The findings of bibliometric analysis confirmed that scientists around the world are trying to analyse of the predictors of the pandemic, predict the consequences and foresight the ways to the recovering after lockdowns. Only the 9 months of the 2020 year the numbers of publication on investigation the pandemic from the medical, economic, and social points of views in the scientific Journal which indexed by Scopus has increased from 300-400 publications in 2019 to 20000-21000 papers. The visualising co-occurrence map allowed concluding that scientists analysed the pandemic under several scientific directions (Fig. 1).

The findings allowed allocating the five scientific directions on the investigation of the pandemic. Thus, the first biggest cluster (red colour) focused on the analysis of the pandemic in relation with public health and socio-economic development issues (social isolation, the income of the society, trust, and awareness). The second cluster (green) focused on the immunology and virology aspects of the pandemic. The third cluster analysed the factors and determinants, which influenced on the pandemic consequences spreading and extending. In the paper [2] the scientists allocated the age as the main factor which influenced the virus penetrating. The fourth cluster (yellow) showed that the scientists analysed the linking between pandemic and air pollutions, climate change, health risks etc.

Noted, that most citable paper (4231 numbers) was published in 2020 in JAMA - Journal of the American Medical Association (Q1) by the Chinese scientists [3]. They presented the clinical findings of the COVID on examples of Wuhan (Chine).

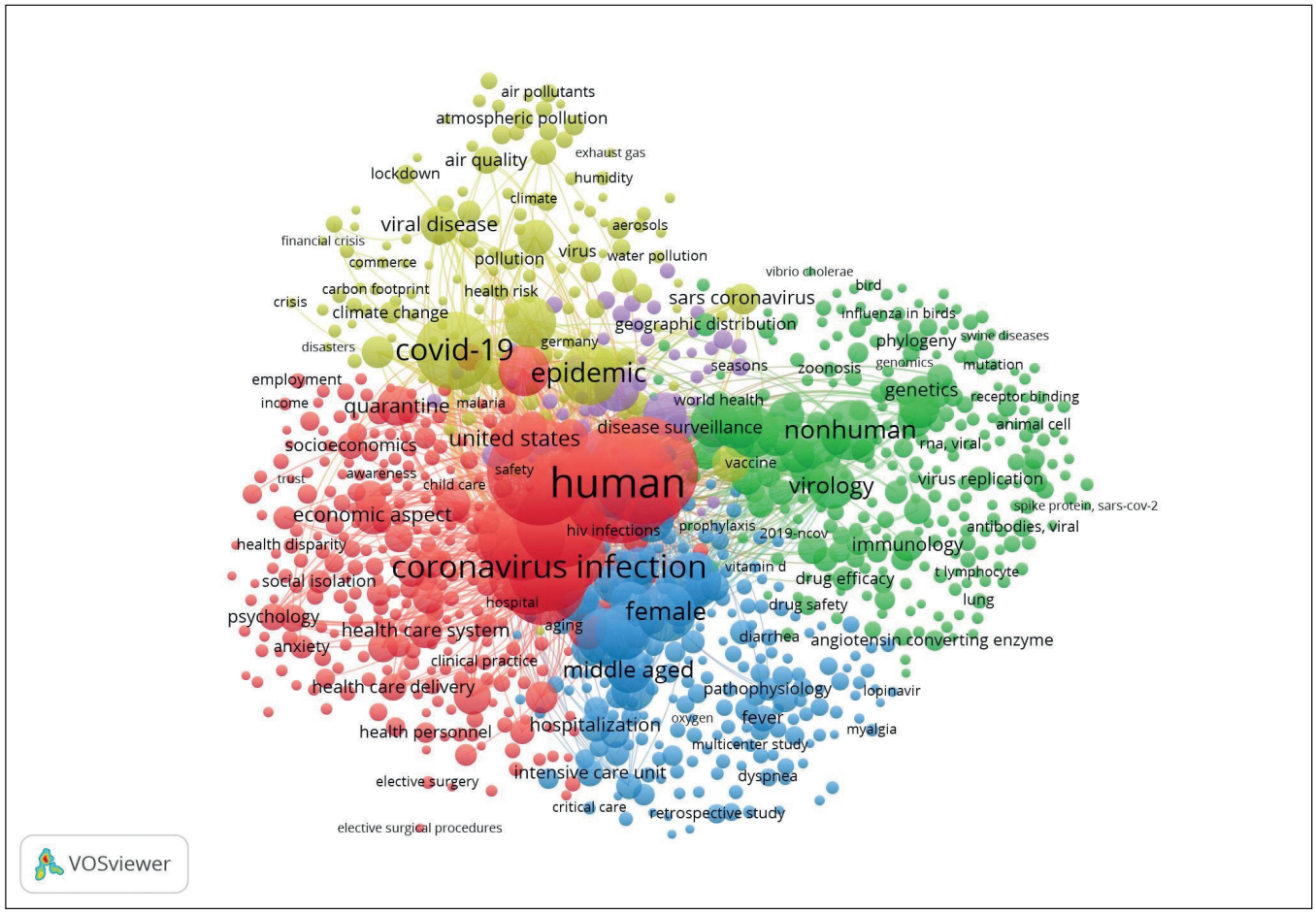


**Fig. 1.** The visualising map of bibliometric analysis of the papers which focused on the investigation of linking between pandemic, air pollution, public health, and economic growth
Source: developed by the authors used VOSviewer and Scopus.

The results of the bibliometric analysis confirmed the increasing interest of the scientists to analyse of the pandemic issues not only from the medical issues [1–3] but also from the social, ecological and economic points of views [4–13]. Besides the vulnerableness of the world under the coronavirus requires the developing of the effective instruments to overcome the recession after the lockdowns. In this case, it is necessary to understand what sphere will be suffered most of all. In order, it requires identifying and empirical justify the linking between the consequences of pandemic lockdown and main economic, social, and ecological parameters.

## THE AIM

The checking the hypothesis on the linking between consequences of pandemic lockdown and air pollution, public health, and economic growth.

## MATERIALS AND METHODS

In the economic theory, the scientists empirical justified the process which relates to the correlation of the economic growth and its social development which measured by the efficiency of supporting and improving the quality of public health. Considering the EKC hypothesis, the economic growth is the instrument to achieve the goals on increasing of quality of public health and protecting of the environment. In this case, the developing countries should provide the policy on economic growth which lead to the achievement of the socioecological and economic goals. At the same time, the isolated government policy-oriented only on environmental protection or only on the social growth limit the economic growth [14]. The graphical interpretation of this hypothesis could be shown as an inverted U-shaped curve (Fig. 2)

In the mathematical expression, the EKC hypothesis could be presented as a function:

$$E=F(Y,Y^2,Z) \quad (1)$$

where E – environmental pollution (per capita GHG emissions), Y – output (per capita GDP), Z – the explanatory variables.

This function derived from the neoclassical theory of economic growth. The Cobb-Douglas production function was the first among all functions. The types of Cobb-Doug-

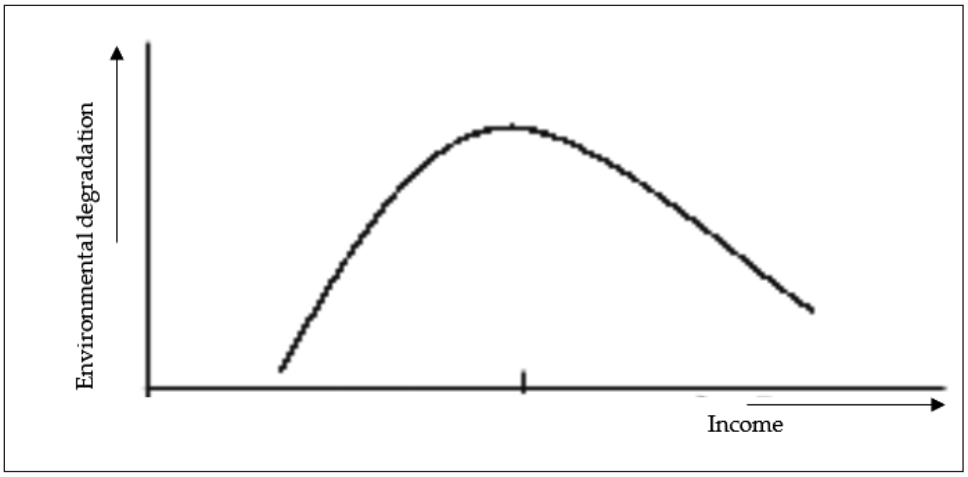


**Fig. 2.** Environmental Kuznets Curve.

las production function use for defining the production volume as the multiplication of production volume and capital. The general function which was developed by the American scientists' Cobb and Douglas in 1928 [15] using the data of economic development of the USA for 1899-1922, showed in formula 2:

$$Q=AL^{\alpha} K^{\beta} \quad (2)$$

where Q – total production (GDP); L – labour costs; K – capital; α i β – coefficients of elasticity, respectively, of capital and labour; A – coefficients of proportionality and scaling, which allows comparing the volume of production from the different time.

At the same time, the global pandemic causes the internal and external shocks through run-on of quarantine which influences on sustainable development of the country. Thus, in the paper [17] the scientists developed the theory on the relations and relationships COVID-19 contagion, government expenditure to the healthcare management and economic indicators of the country development. The authors [17] highlighted the necessity of using the methods of the system dynamics analysis for the justification of the effects of interrelated factors. The similar conclusion was received by the authors in the paper [18]. The authors used the system dynamics approach for modelling the impact of health factors on the economic growth and software VENSIM justified the positive statistically significant impact the social development indicators on the economic growth of the country.

Thus, in the framework of this investigation, the system dynamics analysis was the methodological base for the analysis of the impact of the pandemic lockdown on air pollution, health, and economic growth. It allows considering at the same time, the multiple intersections of different variables of the above concepts in the systems of equations. Besides, the main advantage of this approach is considering of dynamic nature of the process of change which is in the country because of the introduction of quarantine restrictions during COVID 19.

System dynamics analysis include the stage as follows as [19]: developing of the hypothesis of the investigation; identification of the structure and system's modules; causal relationships between them and feedback mechanisms; developing of systems of dynamic equations which emphasis of the processes of change in the social, ecological and economic indicators of the country development; forecasting of social, ecological and economic consequences due to different scenarios of quarantine restrictions using the methods of the system dynamic simulation modelling in Vensim; assessment of the efficiency of the government economic policy during the pandemic.

## RESULTS AND DISCUSSION

At the first stage of System Dynamics Analysis, the system of the dynamic equations could be presented as a variation of relations between variables in the subsequent chain reactions:

(1) GDP → quality of life → population → the number of employed (able-bodied) population; → GDP;

(2) GDP → green investments on declining of air pollution → quality of environment → investment in the fixed assets → GDP;

(3) GDP → investments in the health care system → quality of medical care for patients with COVID 19→ → rate of death → number of employed (able-bodied) population → GDP.

The identified chain interaction parameters allowed developed the casual diagram impact of the pandemic lockdown on air pollution, health, and economic growth (Fig. 3).

The system of the dynamic equations allowed emphasising of the processes of change in the social, ecological, and economic indicators of the country development. The main parameters of the analysis were shown in Table 1.

According to the World Bank Data, economic losses due to a pandemic can be divided into three main categories: about 12% – related to mortality, 28% – due to lack of workers, to 60% –from changes in economic and social behaviour [21]. More recently, the scientific papers and

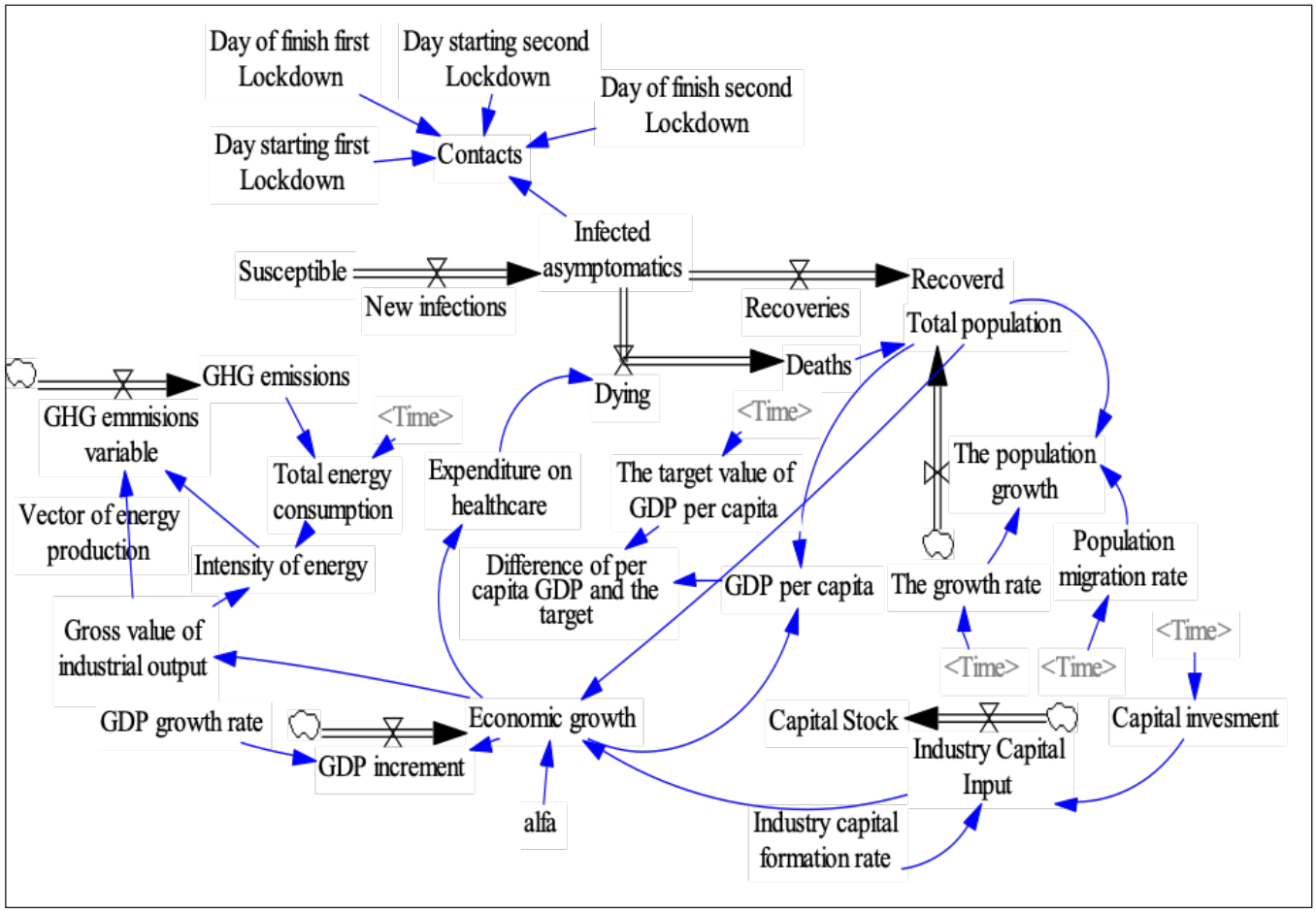


**Fig. 3.** System causalities of the health, ecology, and economic systems.
Note: → – casual impact of the indicators; ⇒► – is a rate variable, a cloud represent a source the starting point of the rate variable.

**Table 1.** Mainly equations in system dynamic model

| Factors | Specific Parameters | Equation |
|---|---|---|
| Environmental factor | GHG emissions | = GDP x Intensity of energy x Vector of Energy Production |
| | Intensity of energy | =Total energy consumption/Gross value of industrial output |
| | Vector of Energy Production | =GHG emissions /Total energy consumption |
| Health and population factor | Susceptible | = Total population – Initial infected |
| | Infected asymptomatic | = Susceptible x New infections |
| | Recovered | = Infected asymptomatic x Recoveries |
| | Death | = Infected asymptomatic x Dying |
| | Expenditure on healthcare | =% GDP |
| | The population growth | = The growth rate x Total population |
| | Total population | = The initial population-Death |
| Economic factor | Economic growth | =(Total population^alfa) x (Industry capital input^(1-alfa)) |
| | The difference of per capita GDP and the target | = GDP per capita-The target value of GDP per capita |
| | The target value of GDP per capita | = providing the annual growing of GDP in average not less than 4% for 2017-2020 years, 6% – for 2021–2025 years and 7% – for 2026–2030 years. |
| | GDP per capita | = GDP/Total population |
| | GDP increment | = GDP growth rate x GDP |
| | Industry capital input | = Industry capital formation rate x Capital Investment |

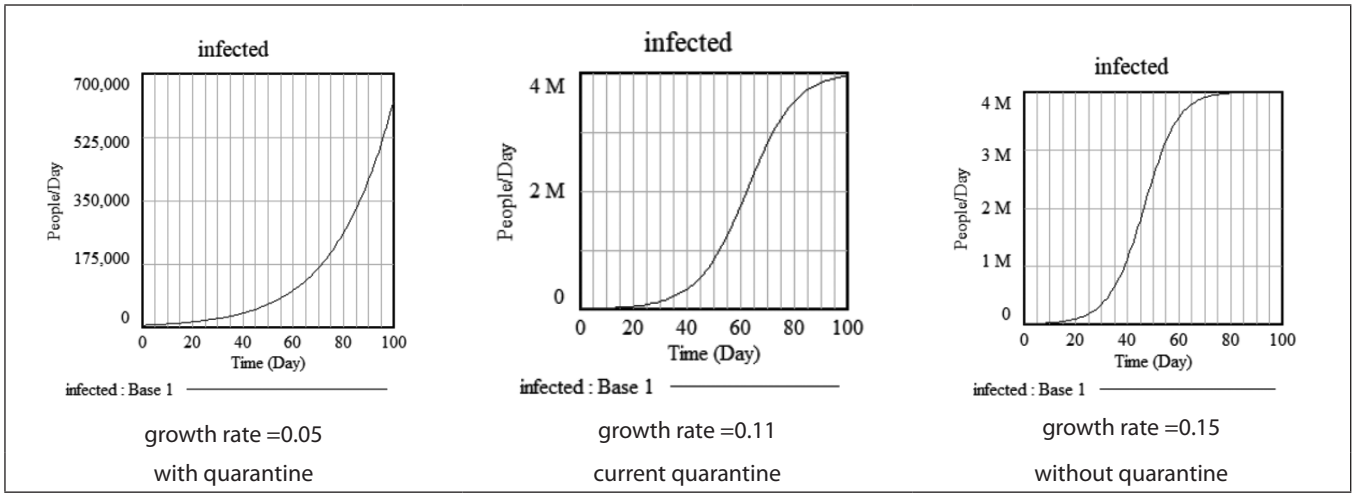


**Fig. 4.** The findings of the system dynamic model the number of infected from growing disease using Vensim

**Table 2.** The findings of alfa calculation using the Ordinary Least Square Methods

| Variable | Coefficient | Std. Error | t-Statistic | Prob. |
|---|---|---|---|---|
| Total population | 0.182586 | 0.089944 | 2.029987 | 0.0574 |
| Industry capital input | 0.935019 | 0.067159 | 13.92243 | 0.0000 |
| R-squared | 0.903628 | Mean dependent var | | 25.31923 |
| Adjusted R-squared | 0.898274 | S.D. dependent var | | 0.543043 |
| S.E. of regression | 0.173201 | Akaike info criterion | | -0.574089 |
| Sum squared resid | 0.539974 | Schwarz criterion | | -0.474516 |
| Log-likelihood | 7.740891 | Hannan-Quinn criteria. | | -0.554651 |
| Durbin-Watson stat | 0.359097 | | | |

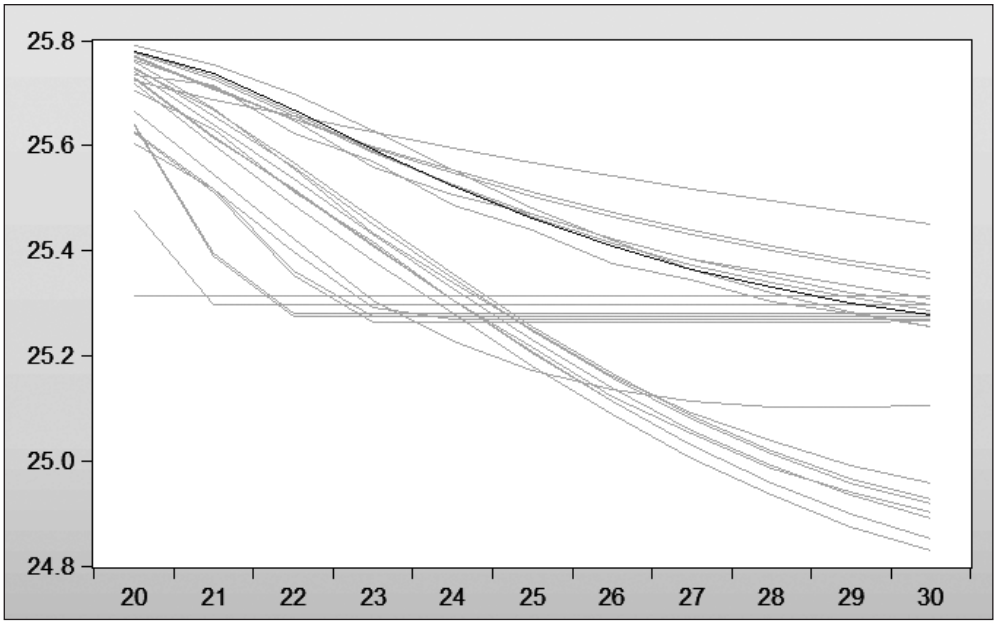


**Fig. 5.** The scenarios of GDP increment changes relate from the quarantine regime

analytical reviews have been published to forecast the COVID-19 pandemic consequences. In the paper [22] the scientists formalised the quarantine impact on the risk of medical system overloading and mortality due to COVID-19. The Institute of Mathematical Machines and Systems Problems of the Ukraine National Academy of Science has developed a determinate SEIR model based on main medical parameters to predict the COVID-19 epidemic extent in Ukraine [23], the results of impact forecasting of social distancing individual scenarios on

the COVID-19 spread are presented in the reports of the World Data Center "Geoinformatics and Sustainable Development" [24], the Institute for Economic Research and Policy Consulting [25].

Considering the OECD report [20] the adaptive quarantine due to COVID-19 has the significant impact on the national economic development. Thus, in the post-COVID-19 the inflation will be 8,7% as in pre-COVID-19 – 5,5%; exchange rate will be increased from 27 (pre-COVID-19) to 29.5 (post-COVID-19). Besides the experts predict the decreasing of GDP by 8% and salary from 12.5 to 10.7 thousand.

The findings of using Vensim after to input data and formulas could be presented as the graphs (Fig. 4). The data confirmed that the current rate of infected from growing disease was 11%. In the government would continue quarantine the rate of infected from growing disease would be 15%. If the quarantine cancelled the rate of infected from growing disease would be 5%. The findings proved the government decisions on prolonging the quarantine regime was one of the effective ways to decline the negative consequences of the COVID-19.

For further calculation, the alfa of the formula (2) as defined by the Ordinary Least Square Methods using the EViews (Table 2).

The empirical results allowed concluding that the Total population and Industry capital input had a statistically significant impact on GDP increment. The coefficient of determination of the model was 90%. Thus, alfa was accepted at the level 0,18.

With the purpose to develop the scenarios of GDP changes under the pandemic lockdown, the forecasting of GDP increment changes was analysed. Thus, the findings of using Vensim after to input data and formulas could be presented as the graph GDP increment (Fig. 5).

The empirical results proved that any types of quarantine regime lead to the declining of GDP increment. Besides, the highest decreasing would be if the government cancel the quarantine regime in Ukraine. Considering the data, the optimal scenario (red line on Fig. 5) – the quarantine would be prolonged.

## CONCLUSIONS

The results of the bibliometric analysis showed the growing interest of the scientists to analyse of the pandemic issues. The scientists try to find effective ways to overcome the recession after the lockdowns due to COVID-19. The empirical results confirmed the hypothesis that lockdown has a negative impact on the economic, social, and ecological growth of the country. At the same time, in the case, if the government cancel the quarantine, the declining of GDP increment will be higher, than in scenario with quarantine. Moreover, the rate of infected from the growing disease will be the lowest if the quarantine will be continued (growth rate – 5%). In this case, the government should provide the quarantine regime and strengthen the control of the compliance.

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ORIGINAL ARTICLE

# MORPHOLOGICAL CHARACTERISTICS OF PLEOMORPHIC ADENOMAS OF SALIVARY GLANDS (ANALYSIS OF THE SURGICAL MATERIAL)

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## ABSTRACT

**The aim** of the study is to reveal the morphological features of pleomorphic adenomas of the salivary glands during a comprehensive examination of the surgical material.

**Materials and methods:** Surgical material from 30 patients with pleomorphic adenomas of the salivary glands was studied. Microspecimens stained with hematoxylin and eosin were studied, using an Olympus BX-41 microscope (Japan) with subsequent processing with the Olympus DP-soft version 3.1 software, which was used to conduct a morphometric study. By morphometry in the tumor tissue, the specific volumes of the parenchyma and stroma, the thickness of the capsule located between the tumor tissue and the tissue of the salivary gland were determined; the absolute number of vessels in the field of view of the microscope was counted at × 100 magnification.

**Results:** Comprehensive morphological analysis of the surgical material of removed neoplasms of the salivary glands has showed that mesenchymal (15 cases, 50.0%) and mixed (10 cases, 33.3%) variants of pleomorphic adenomas are more common, and less often epithelial variants (5 cases, 16.7%). Pleomorphic adenoma is characterized by a different ratio of the epithelial (parenchymal) and mesenchymal (stromal) components forming this tumor, structural diversity and heterogeneity of the structure of these components, which do not have clear boundaries and are mixed with each other. A characteristic feature of pleomorphic adenoma is also the combination in each case of different types of epithelial cells and the structures that they form, as well as areas of various differentiation of the mesenchymal component. Mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant, are more prone to progression and recurrence, as evidenced by our identified active processes of angiogenesis in tumor tissue, frequent tumor invasion of the capsule, thinning of the capsule or the absence of the capsule, less pronounced infiltration of the capsule by immune cells.

**Conclusions:** The morphological features of mesenchymal, mixed and epithelial variants of pleomorphic adenomas of the salivary glands revealed by the authors should be taken into account by clinicians during choosing the tactics for treating the patient, which will undoubtedly help to reduce the incidence of tumor malignization and its recurrence.

**KEY WORDS:** pleomorphic adenoma, salivary gland, morphology

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## INTRODUCTION

Salivary gland tumors, according to the World Health Organization (WHO), represent 3 to 6% of all tumors of the head and neck region, with an annual incidence throughout the world ranging from 0.05 to 2 cases per 100,000 individuals [1]. Epidemiological data reveal different frequencies of salivary gland tumors in distinct ethic groups and geographic locations, which make it difficult to establish global estimates [2].

Pleomorphic adenoma ranks as the commonly occurring benign tumor and constitutes up to two-thirds of all salivary gland neoplasms [3]. Mostly, pleomorphic adenoma is located in the parotid glands (85%), minor salivary glands (10%) and the submandibular glands (5%). In the majority of cases, tumors originate in the superficial lobe. However, occasional cases may involve the deep lobe of the parotid gland and the parapharyngeal space. Minor salivary gland tumors are frequently encountered on the palate, followed by the lip, cheek, tongue and floor of the mouth [4].

The peak incidence of pleomorphic adenoma occurs at a mature age (30-50 years), with women prevailing among the cases (male: female ratio of about 1:1.4) [5]. Pleomorphic adenoma is a tumor with complex histoarchitectonics, characterized by slow growth, scant clinical symptoms and a tendency to recurrence [6]. It has been noted that 50% of recurrences of pleomorphic adenomas of the salivary gland are found in the first two years after surgery. By the end of the five-year follow-up, up to 80% of these neoplasms recur [7].

Clinicians often find it difficult to diagnose pleomorphic adenomas of the salivary glands. This leads to misdiagnosis and, as a result, the choice of the wrong tactics for treating the patient. Errors in diagnosis, according to various authors, range from 7 to 46% of cases. The greatest difficulties arise in the differential diagnosis of neoplastic and reactive-degenerative processes [8].

The most informative, accurate and valuable method of intravital diagnosis of pleomorphic adenoma is morphological examination of biopsy and surgical material. Despite numerous publications of domestic and foreign scientists, interest in the study of the morphological features of pleo-

morphic adenomas of the salivary glands has not weakened until now and remains relevant [9].

## THE AIM

The aim of the study is to reveal the morphological features of pleomorphic adenomas of the salivary glands during a comprehensive examination of the surgical material.

## MATERIALS AND METHODS

Surgical material from 30 patients with pleomorphic adenomas of the salivary glands was studied. The resulting material was fixed in a 10% solution of neutral buffered formalin, carried out according to the generally accepted method and embedded in paraffin. Serial sections 3-4 μm thick were made from paraffin blocks. Microspecimens stained with hematoxylin and eosin were studied, using an Olympus BX-41 microscope (Japan) with subsequent processing with the Olympus DP-soft version 3.1 software, which was used to conduct a morphometric study. By morphometry in the tumor tissue, the specific volumes (%) of the parenchyma and stroma, the thickness of the capsule (μm) located between the tumor tissue and the tissue of the salivary gland were determined; the absolute number of vessels in the field of view of the microscope was counted at × 100 magnification.

Statistical processing of the obtained digital data was carried out, using the Statistica 10.0 program. The means were compared, using the nonparametric Mann-Whitney U-test. Differences were considered significant at $p<0.05$.

## RESULTS

Observational microscopy in all 30 cases revealed fragments of the salivary gland with adjacent tumor tissue, the structure of which corresponded to pleomorphic adenoma. The pleomorphic adenoma in all studied cases was characterized by structural diversity and heterogeneity, which was due, firstly, to the different ratio of the epithelial (parenchymal) and mesenchymal (stromal) components, forming this tumor, and secondly, to the complexity and multicomponent structure of the parenchyma itself and the stroma of the tumor.

The epithelial component in pleomorphic adenomas was represented by epithelial and myoepithelial cells. Epithelial cells were characterized by polymorphism, as they were of different size, shape and nuclear-cytoplasmic ratio. Epithelial cells in most fields of view were round, polygonal, or cubic. Epithelial cells were of the basaloid, spindle-cell, squamous, clear-cell, or plasmocytoid type in part of the visual fields. Less commonly, the epithelial component was represented by mucous, sebaceous, serous or multinucleated cells.

In all 30 cases, clusters of epithelial cells were in the form of nests or strands, which anastomosed with each other (fig. 1, 6). Epithelial cells in 22 cases formed solid, trabecular, cystic, glandular, ductal, or tubular structures (fig. 2, 3). In 4 cases, epidermoid differentiation with the formation of epithelial pearls was revealed in solid beds and ducts (fig. 4).

Myoepithelial cells were few in number, characterized by a focal location and were detected in 14 cases (fig. 3). These cells were located loosely or tightly adjoined to each other, were polygonal or spindle-shaped, sometimes resembling smooth muscle cells.

The mesenchymal (stromal) component of the pleomorphic adenoma in all cases was represented by connective tissue layers or fields with vessels of various sizes located between the fibers. In part of the visual fields, stromal hyalinosis was detected. Vessels in some of the visual fields looked dilated and full-blooded (fig. 5). Diapedetic hemorrhages were observed around some vessels (fig. 5). In all cases, the mesenchymal component of the tumor, in addition to connective tissue, was characterized by the presence of myxoid, chondroid and mucoid zones (fig. 1, 2, 4, 6, 7). In 2 cases, osteoid zones were found in the tumor stroma (fig. 5), and in 1 case – areas of lipomatosis (fig. 6).

It is interesting that in each case, a combination of, first, different types of epithelial cells and the structures that they formed, and, second, areas of different differentiation of the mesenchymal component, was revealed in a pleomorphic adenoma. A characteristic feature of all studied cases of pleomorphic adenomas was absence of clear boundaries between the epithelial and stromal components, mixed with each other.

In all studied 30 cases of pleomorphic adenomas, during morphometry, a different ratio of the specific volumes of the parenchymal and stromal components was noted. As a result, three variants of the tumor were identified. In 15 cases, a mesenchymal variant of pleomorphic adenoma was identified, characterized by a predominance ($p<0.05$) of the stromal component ((84.50±4.41)%) over the parenchymal component ((15.50±4.44)%). In 10 cases, a mixed or classic variant of a pleomorphic adenoma was determined, characterized by the absence ($p>0.05$) of differences between the specific volume of the parenchyma ((53.67±1.49)%) and stroma ((46.33±1.48)%). In 5 cases, an epithelial variant of the tumor was identified, in which the parenchymal component ((87.86±2.16)%) prevailed ($p<0.05$) over the stromal component ((12.14±2.18)%).

When calculating the absolute number of vessels, it was noted that the mesenchymal and mixed variants of pleomorphic adenomas were characterized by a large ($p<0.05$) content of vessels compared to the epithelial variant of the tumor. Thus, the absolute number of vessels in the mesenchymal, mixed and epithelial variants of pleomorphic adenomas was 12.83±1.02, 12.50±0.84, and 8.86±1.02, respectively.

In all cases of pleomorphic adenomas, the morphological features of the capsule located between the tumor tissue and the tissue of the intact salivary gland were assessed during the survey microscopy of the microspecimens and morphometric examination.

On survey microscopy, the capsule consisted of thickened connective tissue fibers with focal infiltration of immune cells, represented mainly by lymphocytes and macrophages (fig. 8). The latter, in part of the visual fields, penetrated the tumor tissue and the tissue of the salivary gland. The interesting thing is that the immune infiltration in the capsule was more pronounced in the epithelial variant of the pleomorphic

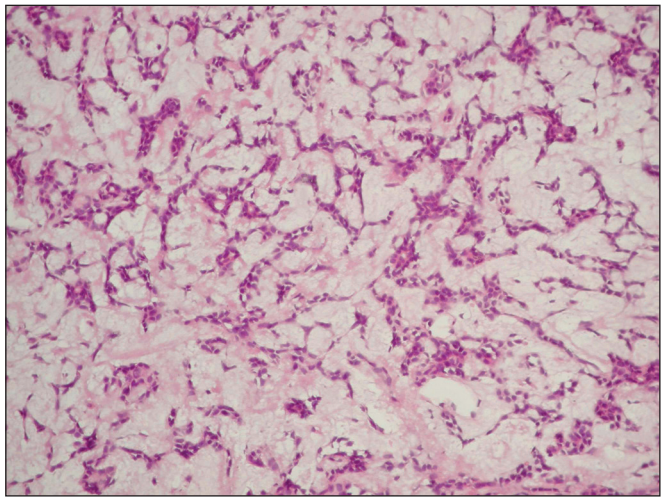

**Fig 1.** Epithelial cells of the tumor form nests and cords that anastomose with each other. The mesenchymal component of the tumor is represented by myxoid and mucoid zones. Stained with hematoxylin and eosin, × 200.

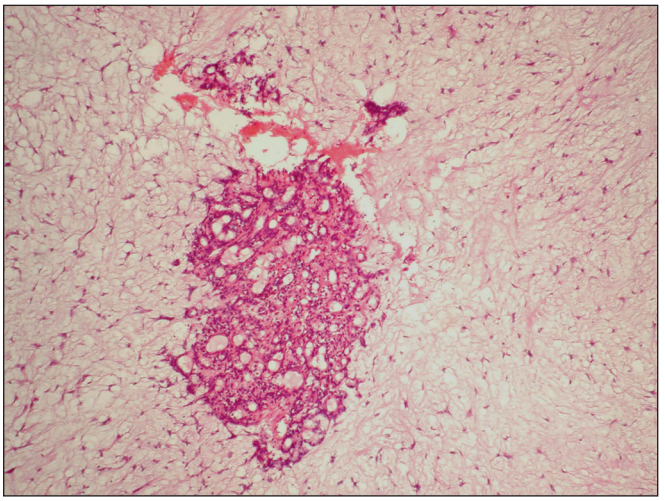

**Fig. 2.** Glandular, ductal and microcystic structures in the tumor parenchyma. The mesenchymal component of the tumor is represented by myxoid and mucoid zones. Stained with hematoxylin and eosin, × 100.

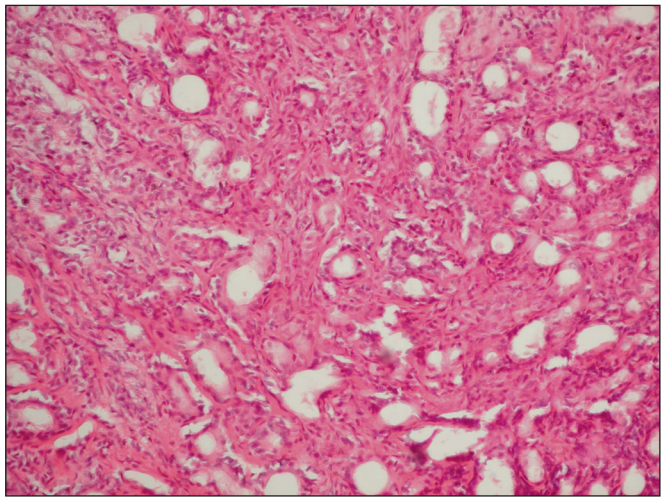

**Fig. 3.** Ductal and glandular structures in the epithelial component of the tumor. Few myoepithelial cells. Stained with hematoxylin and eosin, × 200.

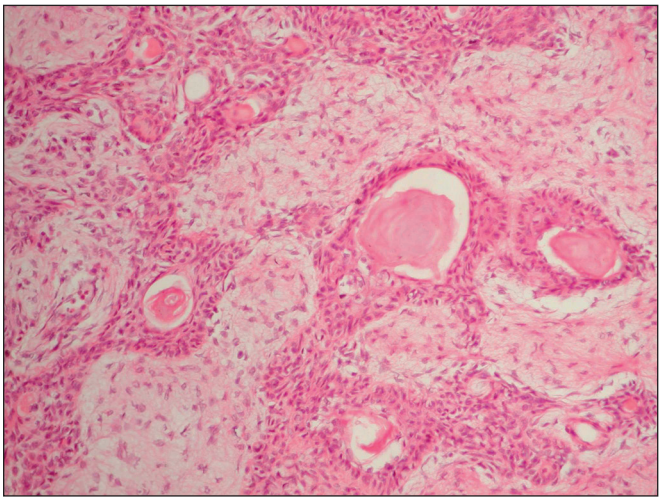

**Fig. 4.** Epidermoid differentiation in the epithelial component of the tumor with the formation of horny cysts. The stroma of the tumor is represented by connective tissue with myxoid and mucoid areas. Stained with hematoxylin and eosin, × 200.

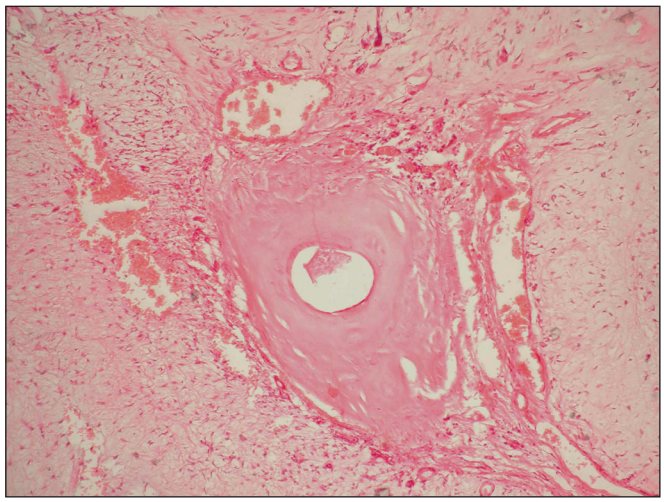

**Fig. 5.** Osteoid area in the tumor stroma. Hyperaemia of the stromal vessels with the formation of diapedetic hemorrhages. Stained with hematoxylin and eosin, × 100.

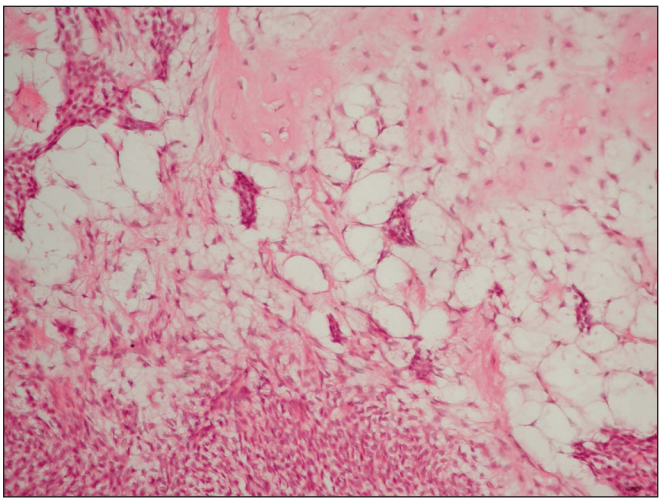

**Fig. 6.** Epithelial component of the tumor in the form of nests and intertwining strands. The mesenchymal component of the tumor is represented by myxoid, chondroid, mucoid zones and areas of lipomatosis. Stained with hematoxylin and eosin, × 200.

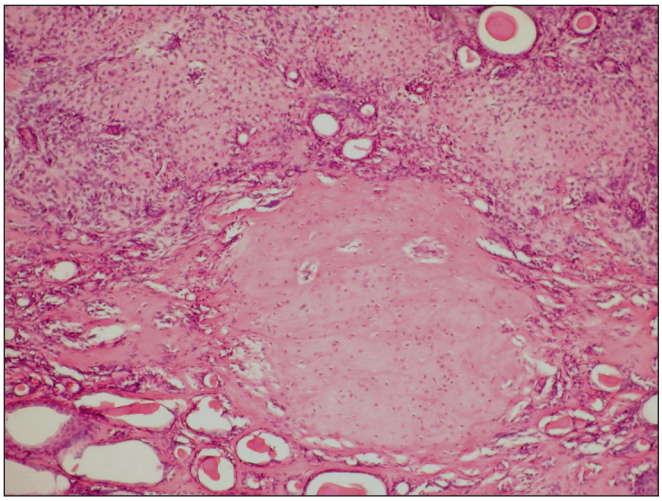

**Fig. 7.** Chondroid sites in the mesenchymal component of the tumor. Stained with hematoxylin and eosin, × 100.

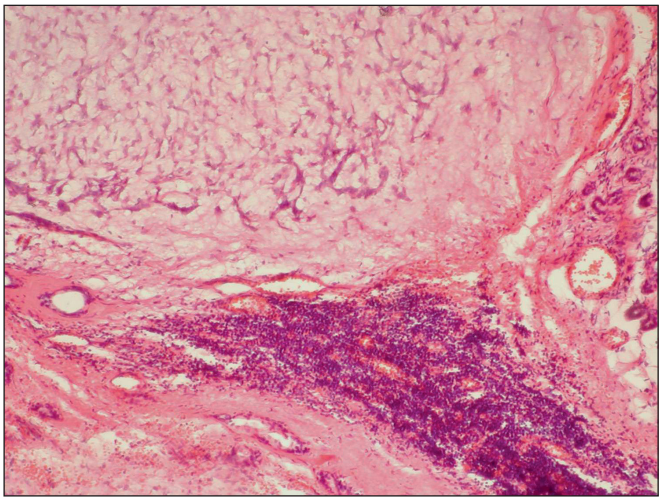

**Fig. 8.** An unevenly expressed connective tissue capsule separating the tumor tissue from the tissue of the salivary gland. Focal infiltration by immune cells of the capsule. Unevenly expressed neoangiomatosis. Stained with hematoxylin and eosin, × 100.

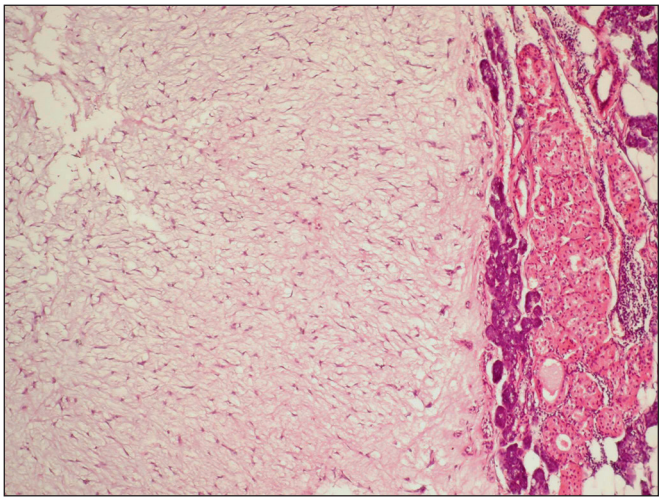

**Fig. 9.** Absence of a capsule between the tumor tissue and the tissue of the salivary gland. Stained with hematoxylin and eosin, × 100.

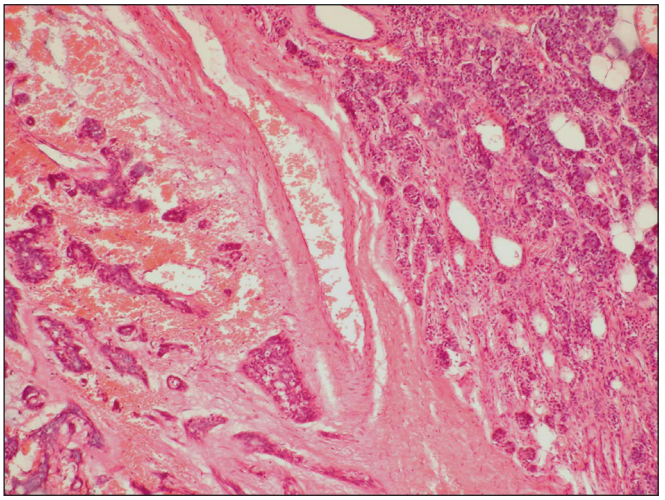

**Fig. 10.** Invasion of the tumor into the connective tissue capsule. Stained with hematoxylin and eosin, × 100.

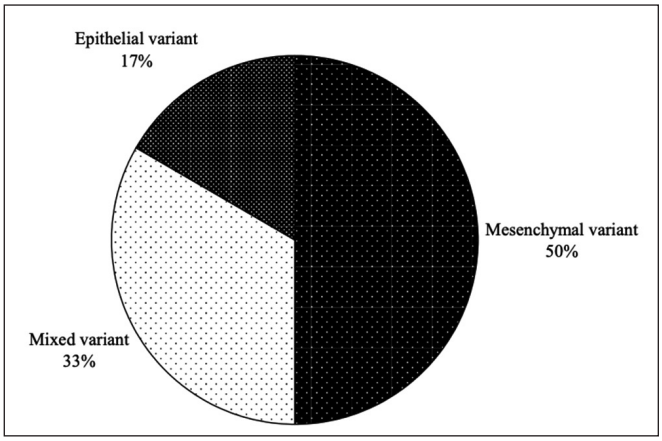


**Fig. 11.** Variants of pleomorphic adenomas among the studied cases.

adenoma, and less pronounced or not at all determined in the mesenchymal and mixed variants of the tumor.

In different variants of pleomorphic adenomas, an analysis of cases with an intact capsule, the absence of the capsule (fig. 9) and tumor invasion into the capsule (fig. 10) was carried out (table 1).

In cases where a capsule separating the tumor tissue from the tissue of the salivary gland was detected during the survey microscopy, its thickness was measured (table 1). It was noted that the mean value of the capsule thickness in the mesenchymal and mixed variants of the tumor was significantly ($p<0.05$) smaller than in the epithelial variant.

## DISCUSSION

It is well known that the human salivary glands, performing protective-trophic, endocrine, excretory and regulatory functions, can be an arena for the development of tumor processes [8]. Pleomorphic adenoma is the most common benign tumor of the salivary glands [6]. Pleomorphic adenoma was first termed by Willis. In the earlier years, it was

**Table 1.** Morphological features of the capsule in different variants of pleomorphic adenomas

| Pleomorphic adenoma variant | Capsule thickness (μm) | Intact capsule | Tumor invasion into the capsule | The capsule was not detected |
|---|---|---|---|---|
| | | Number of cases,% | | |
| Mesenchymal | 6.33±1.32 | 3 cases, 20.0% | 8 cases, 53.3% | 4 cases, 26.7% |
| Mixed | 6.95±1.22 | 2 cases, 20.0% | 4 cases, 40.0% | 4 cases, 40.0% |
| Epithelial | 14.20±1.58 | 4 cases, 80.0% | 1 case, 20.0% | – |

also referred to as mixed tumor, enclavoma, branchioma, endothelioma, endochroma etc [3].

The histogenesis of pleomorphic adenomas of the salivary glands is a controversial issue, which explains the existence of various theories of the origin of the tumor today [10]. The source of pleomorphic adenomas of the salivary glands development can be epithelial cells lining the secretory sections and excretory ducts, as well as myoepithelial cells containing secretory elements in the cytoplasm. The development of this tumor from elements of the stroma is also possible [11]. An important role in the histogenesis of pleomorphic adenomas is assigned to the phenomenon of epithelial-mesenchymal transformation [12].

Pleomorphic adenoma, according to the literature [13] and our study, is characterized by a complex structure. In all 30 cases, it was characterized by a different ratio of its constituent epithelial (parenchymal) and mesenchymal (stromal) components, their structural diversity and heterogeneity, which did not have clear boundaries and were mixed with each other. A characteristic feature also for each case was a combination of various types of epithelial cells and the structures that they formed, as well as areas with different differentiation of the mesenchymal component.

Pleomorphic adenoma of the salivary glands is known to be of four variants. Traditional or classic version, which is characterized by the same content of epithelial and mesenchymal components. The mesenchymal variant of the tumor is characterized by the predominance of the mesenchymal component over the epithelial one. In the epithelial variant of the tumor, the epithelial component predominates over the mesenchymal one. The myoepithelial variant is characterized by a significant content of the myoepithelial component [14].

In our study, among 30 cases, mesenchymal, epithelial and mixed variants of the structure of pleomorphic adenomas were identified (fig. 11). A significant number of cases accounted for mesenchymal and mixed variants of the tumor structure.

Tumors, like normal tissues, need nutrients and oxygen to live, as well as the removal of metabolic products and carbon dioxide. These needs are met by neovascularization – the formation of a network of new blood vessels in the tumor [15].

Active processes of angiogenesis in a tumor, as is known, contribute to its malignancy, the progression of the process, and also increase the likelihood of recurrence [1]. Our survey microscopy, morphometric study with the calculation of the absolute number of vessels in the field of view of the microscope × 100 has showed that the mesenchymal and mixed variants of pleomorphic adenomas are characterized by the highest content of vessels, and the epithelial variant is characterized by the lowest content. The pronounced angiomatous component revealed by us, histogenetically associated with mesenchyme, in mesenchymal and mixed variants of pleomorphic adenomas of the salivary glands, may have an unfavorable prognostic value in terms of malignancy or recurrence of the tumor after its removal.

Benign tumors are known to be characterized by the presence of a well-defined connective tissue capsule. Numerous works have emphasized the barrier function of the capsule. Encapsulated tumors are less aggressive than non-encapsulated tumors [16].

In our study, in the case of mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant of the tumor, more ($p<0.05$) cases with tumor invasion into the capsule and fewer ($p<0.05$) cases with an intact capsule were revealed (table 1). In the case of mesenchymal and mixed variants, cases with the absence of a connective tissue capsule were identified, however, in the epithelial variant of the tumor, such cases were not identified. During morphometry, it was noted that the connective tissue capsule was thinner ($p<0.05$) in mesenchymal and mixed variants of the tumor compared to the epithelial variant. Thus, the analysis of the survey microscopy and the performed morphometric study indicates that the mesenchymal and mixed variants of pleomorphic adenomas are more prone to recurrence compared to the epithelial variant.

Observation microscopy in the connective tissue capsule separating the tumor tissue and intact tissue of the salivary gland revealed focal immune infiltration, which was more pronounced in the epithelial variant of pleomorphic adenoma and less pronounced in the mesenchymal and mixed variants. This infiltration, from our point of view, plays a protective role [17], preventing the invasion of the tumor into the capsule, its progression and recurrence. Thus, the less pronounced immune infiltration in the capsule with mesenchymal and mixed variants of pleomorphic adenomas revealed by us indicates that these variants of the tumor are more prone to invasion into the capsule and penetration into the adjacent tissue of the salivary gland, as well as recurrence.

## CONCLUSIONS

1. Comprehensive morphological analysis of the surgical material of removed neoplasms of the salivary glands has showed that mesenchymal (15 cases, 50.0%) and mixed (10 cases, 33.3%) variants of pleomorphic adenomas are more common, and less often epithelial variants (5 cases, 16.7%).

2. Pleomorphic adenoma is characterized by a different ratio of the epithelial (parenchymal) and mesenchymal (stromal) components forming this tumor, structural diversity and heterogeneity of the structure of these components, which do not have clear boundaries and are mixed with each other. A characteristic feature of pleomorphic adenoma is also the combination in each case of different types of epithelial cells and the structures that they form, as well as areas of various differentiation of the mesenchymal component.
3. Mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant, are more prone to progression and recurrence, as evidenced by our identified active processes of angiogenesis in tumor tissue, frequent tumor invasion of the capsule, thinning of the capsule or the absence of the capsule, less pronounced infiltration of the capsule by immune cells.

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ORIGINAL ARTICLE

# KNOWLEDGE OF YOUNG ADULTS ABOUT NEURODEGENERATIVE DISEASES AND NEUROPROTECTIVE FOOD

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## ABSTRACT

**The aim:** To assess the knowledge of young adults about neurodegenerative diseases and neuroprotective food.

**Material and methods:** The study was conducted using an anonymous self-constructed questionnaire. 150 people aged 18 – 30 participated in the study, including 69% (n = 104) women and 31% (n = 46) men.

**Results:** Most of the respondents had sufficient or good knowledge of neurodegenerative diseases and neuroprotective nutrition. The obtained results did not depend on gender, place of residence, and age. However, knowledge was influenced by education ($p < 0.05$; better-educated respondents indicated more correct answers), and the occupation performed ($p < 0.05$; respondents performing medical professions gave correct answers more often).

**Conclusions:** It seems essential to introduce additional school classes in the field of neurodegenerative diseases and neuroprotective nutrition. Only modern nutritional education from an early age can help implement appropriate eating habits in the field of prevention of neurodegenerative diseases and their application in adulthood.

**KEY WORDS:** neurodegenerative disease, neuroprotective nutrition, questionnaire, knowledge

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## INTRODUCTION

The risk of developing neurodegenerative diseases increases with age [1-3]. We do not yet have effective methods of treating these diseases, but we can delay the aging process of nerve cells and alleviate symptoms, for example, by using a well-balanced diet from an early age, rich in products with neuroprotective properties. Nerve cells are particularly exposed to oxidative stress due to the more significant number of mitochondria and more intense oxygen metabolism. The introduction of products rich in antioxidants into the daily diet allows one to avoid or even reverse oxidative stress. Chemical compounds with antioxidant properties include vitamins A, C, and E, curcumin (present in curry spice and turmeric), polyphenols, e.g., resveratrol (present in red wine and grapes), flavonoids (present in vegetables and fruits, green tea, coffee, cocoa), lycopene (most easily absorbed from processed tomatoes), as well as omega-3 and omega-6 fatty acids. Some of the alkaloids, such as piperine present in black pepper, and capsaicin present in chili peppers, also belong to neuroprotective agents. It is also essential to use products rich in B vitamins (especially B1, B6, B12, folic acid), which condition the nervous system's proper functioning. Increased blood levels of homocysteine have been observed in patients with Parkinson's disease (PD) and Alzheimer's disease (AD). It may be associated with cognitive decline and accelerated deposition of β-amyloid in senile plaques. The sources of the above-mentioned B vitamins are, among others: liver, pistachios, bananas, mandarins, rye bread, soybeans, baker's yeast, broad beans, strawberries, eggs, buckwheat, walnuts, and fish [4-15].

The Mediterranean diet is the most commonly used diet for the prevention and treatment of neurodegenerative diseases. Products of plant origin are the basis of this diet, with simultaneous giving up the consumption of animal products. It is also recommended to use olive oil as the main source of fats, consume red wine moderately, and rely on local, unprocessed products.

## THE AIM

Currently, the nutritional prevention of neurodegenerative diseases is becoming increasingly important. Therefore, this study aimed to assess the knowledge of young people about neurodegenerative diseases and neuroprotective food.

## MATERIALS AND METHODS

The research was carried out using an anonymous proprietary questionnaire. The form was made available on social forums. The questionnaire consisted of 12 single-choice questions concerning knowledge about neurodegenerative diseases and neuroprotective foods, and seven questions concerning gender, age, professional activity, education, place of residence, and family history of neurodegenerative diseases. The study was conducted among young people aged 18 – 30.

The obtained results were statistically analyzed using Excel 2016 and STATISTICA 12.0, Stat Soft Polska software. The result was considered statistically significant if the p was ≤ 0.05. Measurable data were characterized using the mean X and standard deviation SD. Nominal data were presented as percentages.

**Table 1.** Knowledge about neurodegenerative diseases.

| Questions | Possible answers | n (%) |
|---|---|---|
| Characteristic symptoms of Alzheimer's disease | Memory disturbance, failure to recognize loved ones, mood changes, apathy* | 124 (83%) |
| | Mood changes, mobility problems, high blood pressure, constipation | 5 (3%) |
| | Dizziness, pain in the lower abdomen, drooling, constipation | 14 (9%) |
| | I don't know | 7 (5%) |
| Characteristic symptoms of Parkinson's disease | Hand tremor, postural disturbance, changes in handwriting, mask-like face* | 110 (73%) |
| | Problems with concentration, mood disorders, bone, and joint pain, dizziness | 8 (6%) |
| | Difficulty remembering, spatial orientation disorders, muscle tremors, constipation | 26 (17%) |
| | I don't know | 6 (4%) |
| Risk factors for Alzheimer's disease | Older age, male gender, depression | 30 (20%) |
| | Older age, male gender, atherosclerosis | 58 (39%) |
| | Older age, female gender, diabetes* | 38 (25%) |
| | I don't know | 24 (16%) |
| Neurodegenerative diseases include: | Alzheimer's disease, spinal muscular atrophy, Tourette's syndrome | 15 (10%) |
| | Depression, Parkinson's disease, multiple sclerosis | 22 (15%) |
| | Huntington's disease, Alzheimer's disease, Parkinson's disease* | 85 (56%) |
| | I don't know | 28 (19%) |

* The correct answer

## RESULTS

150 people participated in the study, including 69% of 104 women and 31% of 46 men. People aged 18 – 22 (n = 73; 49%) dominated in the study population. More than half of the respondents lived in a city of over 100,000 people (n = 80; 53%). The respondents who had secondary education constituted 48% (n = 72), while those with higher education – 43% (n = 64). In the studied group, more than half had the status of a student (n = 78; 53%), while economically active people accounted for 43% (n = 64). The vast majority of the respondents worked in non-medical professions (n = 101; 67%).

In the families of 71 respondents (47%), no one suffered from any neurodegenerative diseases. Twenty-six respondents (17%) had a family history of Alzheimer's disease, and 15 (10%) had Parkinson's disease. Others were unable to answer this question (26%; n = 38).

One hundred and three respondents (69%) knew the correct definition of neurodegenerative diseases. Twenty-four respondents (16%) answered "I don't know", while 10% (n = 15) believed that this is a group of autoimmune diseases, and 5% (n = 8) – that neurodegenerative diseases belong to the group of civilization diseases.

Answers to questions concerning general knowledge about neurodegenerative diseases are presented in Table 1, and about neuroprotective food – in Table 2.

The respondents received 1 point for each correct answer. The knowledge of the respondents was assessed based on the number of points scored (Table 3).

The results are presented in Table 4. There was no statistically significant difference in knowledge about neurodegenerative diseases and neuroprotective food between men and women. Most of the respondents assessed their knowledge at a sufficient or good level. The knowledge of the respondents also did not depend on the place of residence and age.

On the other hand, the obtained results depended on education ($p < 0.05$; respondents with higher education indicated more correct answers) and profession ($p < 0.05$; respondents from medical professions gave correct answers more often).

## DISCUSSION

Many factors determine the physical and mental well-being of a person. Among them, one can distinguish a well-balanced diet, physical activity, and hygienic lifestyle. To talk about the prevention of neurodegenerative diseases and other neurological diseases, one needs to have a basic knowledge of their symptoms, prevention, and treatment options. Many questionnaire studies assessed Polish society's knowledge of such neurological diseases like stroke or epilepsy [16-20]. Simultaneously, few studies have been done on the knowledge of neurodegenerative diseases and their nutritional prevention [21].

Nutritional knowledge is essential because knowledge of neuroprotective substances and their sources virtually affects the daily diet and determines the use of nutritional prevention of neurodegenerative diseases. Previous work from this center showed that the knowledge of users of online forums about neurodegenerative diseases appeared to be good, while about neuroprotective nutrition, insufficient. Three hundred and seventy people of all ages took part in this study, which was carried out with an online survey. It was proved that the elderly had a higher level of knowledge about the nutritional prevention of neurodegenerative diseases, and the results of their responses

**Table 2.** Knowledge about neuroprotective food.

| Questions | Possible answers | n (%) |
|---|---|---|
| Neuroprotective food include: | Black tea, coffee, saffron | 14 (9%) |
| | Green tea, ginseng, rosemary | 50 (33%) |
| | Ginseng, chocolate, cinnamon* | 36 (24%) |
| | I don't know | 50 (34%) |
| The ingredients that have a positive effect on nerve conduction are: | Stearic acid, taurine | 15 (10%) |
| | Dopamine, serotonin* | 61 (41%) |
| | Acetylcholine, ethanol | 17 (11%) |
| | I don't know | 57 (35%) |
| Products that increase the level of uric acid in the blood | Meat, fish, fructose-rich foods* | 45 (30,0%) |
| | Dairy products, legumes, leafy vegetables | 54 (36%) |
| | Starchy foods, meat, dairy products | 51 (34%) |
| The neuroprotective diet is: | Dukan's diet | 20 (13%) |
| | Cabbage diet | 11 (7%) |
| | High-protein diet | 30 (20%) |
| | Mediterranean diet* | 89 (60%) |
| Products containing curcumin | Curry seasoning* | 90 (60%) |
| | Corn | 7 (5%) |
| | Cardamom | 15 (10%) |
| | I don't know | 15 (10%) |
| | In any of the above | 23 (15%) |
| Factors affecting the formation of free radicals | Smoking, drinking alcohol* | 81 (54%) |
| | Eating plenty of antioxidants | 25 (17%) |
| | Genetic susceptibility to the formation of free radicals | 17 (11%) |
| | I don't know | 27 (18%) |
| The effect of antioxidants on the body | Lowering the risk of developing Alzheimer's disease | 13 (9%) |
| | Slowing down the aging process | 31 (21%) |
| | Prevention of oxidative damage in nerve cells | 23 (15%) |
| | All answers are correct* | 83 (55%) |

* The correct answer

**Table 3.** Rules for assessing the knowledge of the respondents.

| Points | Mark |
|---|---|
| 1-3 | unsatisfactory |
| 4-6 | satisfactory |
| 7-9 | good |
| 10-12 | very good |

**Table 4.** Assessment of the nutritional knowledge of the respondents

| | Mark n (%) | | | |
|---|---|---|---|---|
| Sex | Unsatisfactory | Satisfactory | Good | Very good |
| Males N = 46 | 6 (13.0%) | 15 (32.6%) | 18 (39.1%) | 7 (15.22%) |
| Females N = 104 | 4 (3.9%) | 28 (27%) | 44 (42.3%) | 28 (27%) |

were satisfactory. There was also a statistically significant correlation between the level of knowledge and education. On the other hand, no statistically significant correlation was found between the studied knowledge and gender, place of residence, professional activity, or a positive family history of neurodegenerative diseases [17].

Only people under 30 years took part in this study, conducted three years after the previous assessment. This study aimed to test the knowledge about nutritional prevention of neurodegenerative diseases in young people, bearing in

mind that healthy eating principles should be applied from an early age for the best effect. Most of the respondents assessed the knowledge of neurodegenerative diseases and their nutritional prevention as sufficient or good. As in the previous work, a relationship between knowledge and education was found, while factors such as gender, age, or residence place did not affect the result.

It seems that it would be essential to modify the existing curricula so that information on neurodegenerative diseases, as well as nutrition and prevention of these diseases, would be included in school textbooks, e.g., on biology. This type of knowledge should be disseminated already in the older grades of primary schools and continued in secondary and higher education, both in medical and non-medical students. Only proper nutritional education from an early age can help implement appropriate eating habits to prevent neurodegenerative diseases and their application in adulthood.

## CONCLUSIONS

It seems essential to introduce additional school classes in the field of neurodegenerative diseases and neuroprotective nutrition. Only modern nutritional education from an early age can help implement appropriate eating habits in the field of prevention of neurodegenerative diseases and their application in adulthood.

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**Conflict of interest**

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ORIGINAL ARTICLE

# THE ASSOCIASON OF VITAMIN D RECEPTOR GENE *(VDR)* POLYMORPHISMS WITH HIGH BLOOD PRESSURE IN STROKE PATIENTS OF UKRAINIAN POPULATION

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## ABSTRACT

**The aim:** To study the association of the polymorphisms *VDR* gene with high blood pressure in stroke patients in the Ukrainian population.

**Materials and methods**: Venous blood of 170 patients with atherothrombotic ischemic stroke (AIS) and 124 healthy individuals (control group) was used for genotyping. Four polymorphisms (*Fok*I, *Bsm*I, *Apa*I, *Taq*I) of gene *VDR* were examined with PCR-RFLP methodology. Statistical analysis was performed by using SPSS-17.0 program.

**Results:** The correlation of genotypes of polymorphic variants of *FokI*, *BsmI*, *ApaI* and *TaqI* of the *VDR* gene with the development of ischemic atherothrombotic stroke in individuals with normal and high blood pressure was detected. Statistical analysis of the obtained data revealed that among carriers of genotypes F/F, b/b, a/a, a/A, and T/T patients with AI have statistically significantly higher incidence of hypertension than patients in the control group.

**Conclusions:** It was found that persons with genotypes F/F, b/b, a/a, a/A, and T/T showed a statistically significant relationship between hypertension and the development of IAS. The application of logistic regression has made it possible to establish that the risk of IAS in people with normal blood pressure and genotype F/f is 3.2 times higher than in normotensive homozygotes for the F-allele.

**KEY WORDS:** high blood pressure, ischemic stroke, gene polymorphism, *VDR*

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## INTRODUCTION

Brain lesions are one of the most pressing problems of modern medicine and are among the main factors causing death and disability of the planet's population [1, 2]. Ischemic stroke is a complex disease with wide variations in the age at onset, etiology, comorbidities, and adequacy of collateral circulation. BP is a simple physiologic parameter that is always measured, can be modulated, and may affect the outcome in certain circumstances [3]. All over the world, a lot of attention to a problem of association between allelic gene polymorphism and development of most widespread pathological processes and human diseases has been paid. Recently genes, which depend on the intensity and direction of the calcium-phosphorus metabolism in the body as a whole and in certain tissues, among factors that influence the damage of blood vessels have been named. These includes vitamin D receptor gene (*VDR*). Furthermore, according to clinical trials, there is an inverse correlation between low level of vitamin D and risk factors, such as blood pressure (BP), coronary atherosclerosis and various cardiovascular diseases, arterial hypertension in particular [4, 5].

With the development of molecular genetic technologies, there is many opportunities to study the genetic component of cerebrovascular pathology.

Around the world, attention to the problem of linking allelic gene polymorphism with the development of the most common pathological processes and human diseases is drawn. Among the many polymorphisms studied in these laboratories are genes associated with diseases such as hypertension, coronary heart disease, obesity, acute coronary syndrome, ischemic stroke. One of the genes whose only variants are being studied by scientists is the vitamin D receptor gene. It is likely that there may be a link between vitamin D receptor gene (VDR) polymorphism and acute cerebral circulatory disorders since there are a number of related factors. Vascular wall changes exert its effect through interaction with the vitamin D receptor [6]. The effective activity of this system may depend on many factors, including the polymorphism of the genes encoding the structure of the respective proteins. In turn, VDR encoded by a gene characterized by genetic polymorphism, that is, the existence of different allelic variants of this gene in the population [7]. The most significant VDR gene polymorphisms involved in disease development are *Fok*I, *Bsm*I, *Apa*I, *Taq*I [128, 294]

The lack of data on the association of vitamin D receptor gene polymorphism with ischemic atherotrombotic strokes (IAS) and its developmental factors, namely arterial hypertension not only in Ukrainian but also in other populations is another factor that has prompted our own research.

## THE AIM

Aim of our study was the association of the polymorphisms VDR gene with hypertension in stroke patients in the Ukrainian population.

## MATERIALS AND METHODS

*Subjects*. The studied group included 170 patients with IAS (42.4% women and 57.6% men) aged 40 to 85 years (mean age 64.7 ± 0.73 years). The ischemic nature of the stroke according to the anamnesis and clinical picture of the disease, the results of an MRI study of the brain was established. The pathogenic variant of stroke was determined according to TOAST criteria [8], based on the anamnesis and features of the clinical course of the disease, ultrasound Doppler ultrasound, ECG. The control group consisted of 124 patients in whom the absence of cardiovascular pathology was confirmed by anamnestic data collection, electrocardiogram withdrawal and blood pressure measurements. The control group and the group of patients with IAT did not differ in the ratio of persons of different sex (P = 0.294 on the $\chi 2$ criterion), but the mean age of the first (76.7 ± 0.93 years) was significantly higher than the second was (P <0.001) [9].

*Amplification and genotyping*. DNA for genotyping from the venous blood using commercially available kits (Isogene Lab Ltd, Russia) according to the manufacturer's protocol was extracted. Determination of polymorphisms *Fok*I- (rs 2228570), *Bsm*I- (rs1544410), *Apa*I- (rs7975232), and *Taq*I- (rs731236) of the *VDR* gene using the polymerase chain reaction method followed by restriction fragment length analysis upon detection by agarose gel electrophoresis was performed. Primers synthesized by Metabion (Germany) (Table 1) and enzymes (Taq polymerase and restrictase) by Thermo Scientific (USA) were used. PCR in a GeneAmp PCR thermocycler System 2700 ("Applied Biosystems", USA) was performed.

Detection of the restriction products by horizontal electrophoresis in 2.5% agarose gel (Sigma-Aldrich, USA) was performed containing ethidium bromide (Sigma-Aldrich, USA). The results in ultraviolet rays using an automatic video reading system "Vi-Tran" in a transilluminator ("Biocom" Russia) were visualized [9].

*Statistical analysis*. Statistical analysis was performed using SPSS-17. Before testing the statistical hypotheses, an analysis of the normality of the distribution of values in the samples was carried out, by determining the asymmetry and excess coefficients using the Willkie-Khan-Shapiro and Lilliefors criteria using the algorithms implemented in SPSS-17. The significance of the differences between the two samples was determined using Student's t test (t). Based on the magnitude of t and the number of degrees of freedom (l = n1 + n2-2), the difference between the two samples (P) was found on the Student's distribution table. The difference was considered significant if the probability of a random difference did not exceed 0.05 (p <0.05). Non-parametric criteria to estimate differences in mean trends and independent samples were used, namely the Fisher exact method for a four-field table (TMF). The use of nonparametric criteria made it possible to find out significant differences in cases where the criterion t did not reveal them.

## RESULTS

Analyzing patient groups formed based on genotype *Fok*I polymorphisms on VDR gene (F/F, F/f, f/f), the following data were obtained. Among the carriers of the genotype F/F in the control group was 48.5% of individuals with normal blood pressure and 51.5% of those with hypertension, and in the group of patients with IAS 15% and 85% respectively. Statistical analysis of the data shows that there is a statistically significant correlation between the level of blood pressure and the probability of development of IAS in the homozygote F/F: in patients with arterial hypertension, IAS was found more frequently than in patients with normal blood pressure ($\chi 2 = 9.629$, $P_1 = 0.002$). Among individuals with the F/f genotype, 32.8% of subjects with normal blood pressure and 67.2% of persons with high blood pressure were in control group, and in the group of patients with IAS, their number was 32.8% and 74.7%, respectively. There was no significant difference in the incidence of subjects with normal and increased blood pressure with the F/f genotype in the comparison groups ($\chi 2 = 0.980$, $P_2 = 0.322$). As for carriers' f/f genotype, the control group had 43.3% of those with normal blood pressure and 56.7% with hypertension and among patients with IAS – 33.3% and 66.7% respectively. The frequency of those carriers f/f genotype among people with normal and high blood pressure in the control and experimental group does not get beyond statistical significance ($\chi 2 = 0{,}722$, $P_3 = 0.395$) (Table 2).

**Table 1.** Conditions for PCR and restriction analysis

| SNP | The nucleotide sequence in the primers | Restriction enzime | Restriction fragments, b.p. |
|---|---|---|---|
| *Fok*I rs2228570 | Fw 5′-AGCTGGCCCTGGCACTGACTCTG-3′ Rev 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′ | *Fok*I | 267, 204, 63 |
| *Bsm*I rs1544410 | Fw 5′-AGGGAGACGTAGCAAAAGGAG-3′ Rev 5′-TGTCCCCAAGGTCACAATAAC-3′ | *Bsm*I | 425, 232, 193 |
| *Apa*I rs7975232 | Fw 5′-CAGAGCATGGACAGGGAGCAA-3′ Rev 5′-CACTTCGAGCACAAGGGGCGTTAGC-3′ | *Apa*I | 501, 284, 217 |
| *Taq*I rs731236 | Fw 5′-CAGAGCATGGACAGGGAGCAA-3′ Rev 5′-CACTTCGAGCACAAGGGGCGTTAGC-3′ | *Taq*I | 501, 294, 207 |

**Table 2.** Distribution of individuals of different genotypes for VDR gene polymorphisms in the control group and the group of patients with IAS depending on the size of the arterial pressure

| SNP | Genotype | Arterial hypertension | Ischemic stroke n (%) | Control group n (%) | P |
|---|---|---|---|---|---|
| *Fok*I | F/F | – | 6 (15.0) | 16 (48.5) | 0.002 (1) |
| | | + | 34 (85.0) | 17 (51.5) | |
| | F/f | – | 23 (25.3) | 19 (32.8) | 0.322 (2) |
| | | + | 68 (74.7) | 39 (67.2) | |
| | f/f | – | 13 (33.3) | 13 (43.3) | 0.395 (3) |
| | | + | 26 (66.7) | 17 (56.7) | |
| *Bsm*I | b/b | – | 16 (22.5) | 23 (41.1) | 0.025 (4) |
| | | + | 55 (77.5) | 33 (58.9) | |
| | b/B | – | 19 (25.7) | 19 (37.3) | 0.167 (5) |
| | | + | 55 (74.3) | 32 (62.7) | |
| | B/B | – | 7 (28.0) | 6 (42.9) | 0.345 (6) |
| | | + | 18 (72.0) | 8 (57.1) | |
| *Apa*I | a/a | – | 7 (15.6) | 17 (43.6) | 0.005 (7) |
| | | + | 38 (84.4) | 22 (56.4) | |
| | a/A | – | 22 (25.9) | 21 (42.0) | 0.052 (8) |
| | | + | 63 (74.1) | 29 (58.0) | |
| | A/A | – | 13 (32.5) | 10 (31.2) | 0.910 (9) |
| | | + | 27 (67.5) | 22 (68.8) | |
| *Taq*I | T/T | – | 15 (22.1) | 21 (39.6) | 0.036 (10) |
| | | + | 53 (77.9) | 32 (60.4) | |
| | T/t | – | 22 (26.8) | 21 (38.9) | 0.139 (11) |
| | | + | 60 (73.2) | 33 (61.6) | |
| | t/t | – | 5 (25.0) | 6 (42.9) | 0.273 (12) |
| | | + | 15 (75.0) | 8 (57.1) | |

**Table 3.** Risk analysis IAS depending on genotype FokI polymorphism of VDR gene in patients with normal and high blood pressure (logistic regression method)

| | Genotype | CR | SE | WS | P | OR | 95% CI for OR lower | 95% CI for OR upper |
|---|---|---|---|---|---|---|---|---|
| Normal BP | F/f | 1.172 | 0.570 | 4.222 | 0.040 | 3.228 | 1.056 | 9.872 |
| | f/f | 0.981 | 0.619 | 2.512 | 0.113 | 2.667 | 0.793 | 8.969 |
| Arterial hypertension | F/f | 0.137 | 0.359 | 0.146 | 0.702 | 0.872 | 0.432 | 1.761 |
| | f/f | 0.268 | 0.431 | 0.388 | 0.533 | 0.765 | 0.329 | 1.779 |

Note: Homozygotes for f-allele (f/f) are compared with carriers of F-allele (F/f+F/F). CR – regression coefficient, SE – standard error, WS – Wald statistics, P – statistical significance, OR – risk ratio, CI – confidence interval.

Application of logistic regression method made it possible to establish that the risk of occurrence of IAS in persons with normal blood pressure and genotype F/f is 3.2 times higher than in normotensive homozygotes according to F-allele (Table 3).

The study of the frequencies of persons with normal and high blood pressure in the comparison groups depending on the variants of the genotype according to the *Bsm*I-polymorphism of the *VDR* gene was informative. Thus, among the carriers of the b/b genotype, 41.1% of persons with normal pressure and 58.9% with high blood pressure in the control group and 22.5% and 77.5% in the group of patients with IAS were detected respectively. Statistical analysis of the data revealed that among carriers of the b/b genotype, patients with IAT have a significantly higher frequency of hypertension than patients in the control group ($\chi 2$ = 5,055, $P_4$ = 0.025). Among persons with the b/B genotype, 37.3% of those with normal pressure and 62.7% with high blood pressure were in control, and in the group of patients with IAS, their number was 25.7% and 74.3%, respectively. There are no differences in the frequencies of individuals according to this genotype in the comparison groups

($\chi2$ = 1.913, $P_5$ = 0.167). Regarding carriers of genotype B/B, 42.9% of subjects with normal pressure and 57.1% with hypertension in the control group and among patients, there were 28.0% and 72.0% were found respectively. Frequency of carriers of genotype B/B among representatives with different values of blood pressure in the control and experimental groups does not get beyond statistical significance ($\chi2$ = 0,891, $P_6$ = 0,345) (Table 2). Therefore, in homozygotes for the b-allele and not the B-allele carriers, patients with IAT have a higher incidence of hypertension than patients in the control group.

In the analysis of groups of patients formed based on the genotype by the *Apa*I polymorphism of the VDR gene (a/a, a/A, A/A), the following data were obtained. Among the carriers of the genotype a/a in the control group, there were 43.6% of persons with normal and 56.4% of persons with high blood pressure, and in the group of patients with IAS 15.6% and 84.4% respectively. Statistical analysis of the data indicates that homozygotes a/a have a statistically significant relationship between the level of blood pressure and the likelihood of developing IAS: in individuals with arterial hypertension IAS was found more often than in patients with normal blood pressure ($\chi2$ = 8,046, $P_7$ = 0,005). There were 42.0% of subjects with normal BP and 58.0% with increased BP among control group with the A/A genotype, and in the IAS group, their numbers were 25.9% and 74.1%, respectively. Therefore, the incidence of subjects with normal and increased blood pressure with a/A genotype in the comparison groups is statistically significant. ($\chi2$ = 3.768, $P_8$ = 0.052). Regarding A/A genotype carriers, 31.3% of subjects with normal blood pressure and 68.8% with hypertension was in the control group, and among patients with IAS 32.5% and 67.5% were found respectively. The frequency of A/A genotype carriers among patients with normal and elevated blood pressure in the control and study group was not statistically significant ($\chi2$=0.013, $P_9$ = 0.910) (Table 2).

Study of frequencies of persons with normal and high blood pressure in comparison groups depending on variants of the genotype by *Taq*I-polymorphism of the VDR gene was informative too. Thus, among the carriers of the T/T genotype, 39.6% of persons with normal pressure and 60.4% with high blood pressure were detected in the control group, and 22.1% and 77.9% respectively in the group of patients with IAS. Statistical analysis of the data revealed that among carriers of the T/T genotype, patients with IAS have a significantly higher incidence of hypertension than patients in the control group ($\chi2$ = 4.396, $P_{10}$ = 0.036). Among those with the T/t genotype, 38.9% had normal pressure and 61.1% had increased blood pressure in the control group, and 26.8% and 73.2% in the IAS group respectively. There were no differences in the frequencies of individuals according to this genotype in the comparison groups ($\chi2$ = 2,190, $P_{11}$ = 0,139). As for carriers of t/t genotype, in the control group 42.9% of those with normal blood pressure and 57.1% with hypertension and among these patients with IAS 25.0% and 75.0 % were found respectively. The frequency of carriers of the genotype t/t among representatives with different values of blood pressure in the control and experimental group does not go beyond statistical significance ($\chi2$ = 1,200, $P_{12}$ = 0,273) (Table 2). Therefore, in T-allele homozygotes and not in the t-allele carriers, patients with IAS have a higher incidence of hypertension than patients of the control group.

## DISCUSSION

Worldwide, ischemic stroke accounts for 85-90% of all strokes, of which 60% is associated with the development of hypertension, diabetes, heart disease, high cholesterol, obesity and smoking. Another 40% of atherothrombotic ischemic stroke, about half are associated with other acquired pathologies and the other half is caused by unknown factors [10]. Determining the role of a particular gene in the development of ischemic stroke is challenging. This is due to its interaction with other genes and factors, as well as with related diseases [11]. Thus, there is an increase in the risk of disease was associated with the carrier of one gene in combination with other genes whose actions are synergistic with respect to the risk of ischemic stroke [11, 12]. It is important to note that there is a genetic heterogeneity of ischemic strokes or, in other words, each clinical and pathogenic variant of a stroke has unique gene combinations. The risk of ischemic stroke increases not only under the influence of polymorphism of one gene, but under also when alleles of several genes are combined, that is, there is a polygenic hereditary predisposition to thrombotic lesions of brain vessels is established [12].

VDR exercises its influence on the development of hypertension using different mechanisms. However, the most important of these is influenced by VDR on the process of calcification of the vascular wall through the regulation of Matrix Gla-Protein synthesis [13-15].

In our study, the assosiation between the *Fok*I-, *Bsm*I-, *Apa*I-, and *Taq*I-polymorphisms of the *VDR* gene with the development of IAS in individuals with normal and increased blood pressure have been identified. When analyzing groups of patients formed with the FokI polymorphism genotype among carriers of the F/F genotype, there is a statistically significant relationship between the level of the blood pressure and the probability of IAS development [16-18].

The data we obtained are consistent with those of other sources [19]. In patients with hypertension with the genotypes F / F, F / f, compared with patients with the genotype f / f, were recorded higher values of daily average $AP_{syst}$ and average $AP_{syst}$ per day was reported.

Shuai Lu, et al. informed that the Fok1 polymorphism may play a protective role in Coronary artery disease (CAD), and the possible protective role in Apa1 CA genotype in CAD patients with T2DM needs further studies. The Taq1 polymorphism is found to be associated with a significant increase in CAD risk based on our analysis; moreover, increased risk in Apa1 polymorphism in CAD patients without T2DM and Bsm1 polymorphism in Caucasian group is also detected [20].

## CONCLUSIONS

It was found that persons with genotypes F/F, b/b, a/a, a/A, and T/T showed a statistically significant relationship between hypertension and the development of IAS. The application of logistic regression has made it possible to establish that the risk of IAS in people with normal blood pressure and genotype F/f is 3.2 times higher than in normotensive homozygotes for the F-allele.

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ORIGINAL ARTICLE

# COMPARATIVE MORPHOLOGY OF THE PIG`S RECTUM AND HUMAN`S RECTUM VIA 3D RECONSTRUCTION

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## ABSTRACT

**The aim:** To define an ability to use pig as biomodel for experimental and clinical studies in order to develop new approaches of treatment of the rectal pathology.

**Materials and methods:** For the research two groups were used. I group includes 50 humans (27 females and 23 males), II group includes 8 vietnamese pot-bellied pigs. After magneresonance Imaging the 3D reconstruction of rectum was made.

**Results and conclusions:** Topography, structure, age and gender peculiarities of pig`s and human`s rectum in comparative aspect are described in this article. With the help of modern methods, namely 3D reconstruction, the structure of the pig`s and human`s rectum was reconstructed. The morphological parameters, such as size of anorectal and rectosigmoid angles, the length of the rectum and its parts, were determinated.

The 3D reconstruction demonstrated that size of anorectal and rectosigmoid angles, the length of the pig`s rectum are morphologicaly identical to the same parametres of human`s rectum. Thus, it is proved that pigs can be used as biomodels in experimental and clinical studies for development the new methods of treatment the rectal pathology in humans.

**KEY WORDS:** anorectal angle, rectosigmoid angle, biomodel

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## INTRODUCTION

The significant progress has been made in the treatment of rectal diseases via using the modern techniques over recent years. The result has been reduction of frequency of complication such as purulent-inflammatory complications, an anal incontinent and relapses [1, 2, 3, 4]. Despite modern advances in surgical treatment of rectal pathology, a sufficiently high frequency of unsatisfactory treatment results remains, such as: relapsing fistulas of the rectum are observed in 35 – 40,2% of patients, purulent-inflammatory complications 13 – 20% of patients, there is an anal incontinent in 19 – 23% of patients [5-9]. Such results show that need to revise the date of rectum structure and the improvement of biological model to develop new approaches in rectum treatment. Nowadays laboratory rats are a traditional model of experimental researches. But considering the size of rat`s rectum, the process of defecation, the rats are not appropriate model for formation surgical skill during practical classes, conducting manipulation, especially for creation modern surgical approaches for prevention and treatment rectal diseases. Modern experiences of leading medical institutions in Europe, the USA and Japan publish researches in the field of xenotransplantation, reconstruction of mechanism of human diseases at the molecular level, such as Duchenne Muscular Dystrophy, cystic fibrosis with using of pigs as biomodels. [10-14]. Swine as a biomodel is anatomically and physiologically more similar to humans, especially regarding cardiovascular system, immune system, respiratory system, skeletal muscle, metabolism, etc. [15]. The pig as a biological model is actively used in researches of medical industry, namely medical technologies (instruments, apparatus etc.).

In this regard, the actual task is to determine the feasibility of the use of pigs as biomodels of various diseases and the use of this animal in the medical field generaly.

## THE AIM

To define an ability to use pig as biomodel for experimental and clinical studies in order to develop new approaches of treatment of the rectal pathology.

## MATERIALS AND METHODS

The research was conducted in two stages. The first stage provided for clinical study. Healthy people were attracted (I group – 27 females and 23 males), adults, who agreed to participate in a clinical study. After anoscopy and rectoroscopy, the pathology of the rectum was excluded. The magneto-resonance tomography was made with the subsequent 3-D reconstruction and morphometry.

All clinical procedures were carried out in compliance with the main provisions of GCP (1996), the Council of Europe

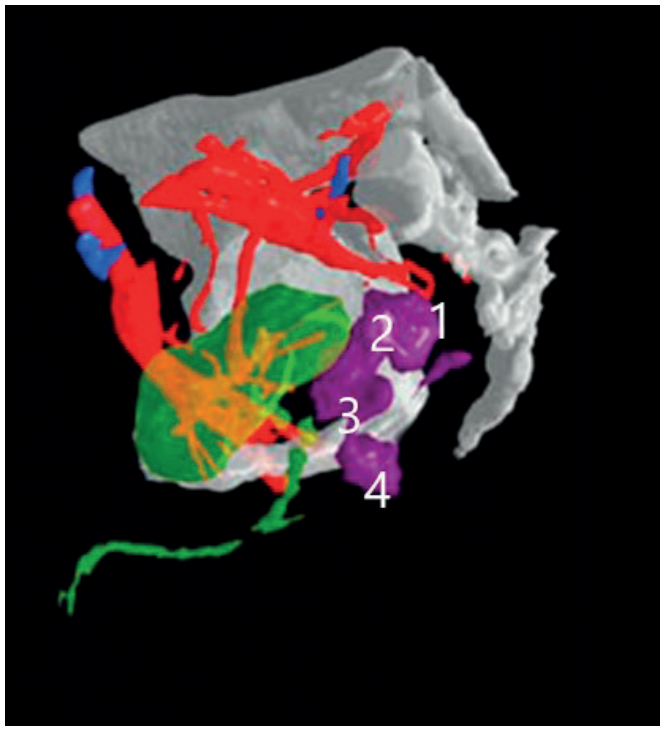


**Fig.1.** Lateral view of male pelvis.
1- Rectosigmoidal angle. 2- Pelvis part of rectum. 3- Anorectal angle. 4- Anal canal.

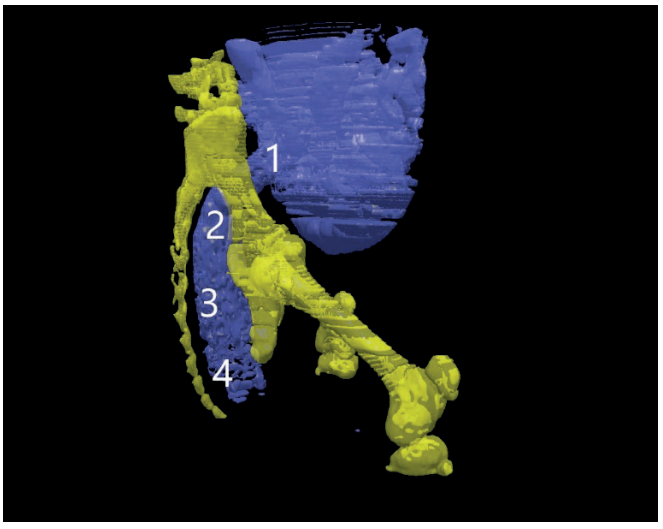


**Fig. 2.** Lateral view of pig`s pelvis.
1- Rectosigmoidal angle. 2- Pelvis part of rectum. 3- Anorectal angle. 4- Anal canal.

Convention on Human Rights and biomedical (04.04.1997), the Helsinki Declaration of the World Medical Association on the Ethical principles of conducting scientific medical research involving the person (1964-2013), the order of the Ministry of Health of Ukraine №690 (23.09.2009), №616 (03.08.2012).

The second stage of research was the experimental stage with using of vietnamese pot-bellied pigs as a subresearch animal. The experiment used 8 castable males of 5 month age and an average weight of 11-11.3 kg. Conditions of maintenance of experimental animals conform to current norms of Ministries of agrarian policy of Ukraine. The feeding of animal was corresponded to needs in high-quality and biologically active substances. The eperiment was carried out in a specially equipped veterinary laboratory, provided by all necessary equaipment for interventions and individual observation of animals. The high-qualified employers of Scientific center "Vetmedservis" were involved in the work with animals. For all animals after the confirmation of animal health by veterinarian the blood test and anoscopy were done. After sedation to the group of animals the magneresonance imaging was held. The study was conducted in compliance with the current legislation of Ukraine, the Council of Europe Convention on the Protection of vertebrate animals used in experiments and other scientific purposes (18.03.1986), the EU Directive №609 (24.11.1986).

For 3D reconstruction the graphical Wacom manipulator was used. Via the superfacial rendering the anatomical structures of every slide were marked with different color. This methodic allowed to clearly present their shape, interlocation, relative dimensions, etc. In addition, this made it possible to accurately conduct morphometry – to determine the dimensions, angles, length.

## RESULTS AND DISCUSSION

Due to the using morphometry, the parameters of the rectum such as anorectal angle and the rectosigmoid angle, of the I clinical group and experimental animals of the II group were defined (Tabl. 1).

The noteworthy feature, that 3D remodeling can not display structural elements of mucous coat of rectum, we use rectosigmoid angle and anorectal angle as points of the start and finish of rectum.

According to the 3D reconstruction during our research, it was determined that rectosigmoid angle is located at the level of the second sacral vertebra in both groups and equal to 131 ± 0,7 °C (females of I group), 130 ± 0,4 °C (males of I group) and to 140±1 °C (I group).

Detected that distance between anorectal angle and rectosigmoid angle, means pelvic part of rectum is equal to 130±3 mm (males of I group) and 133±2 mm (females of I group), 170±5 mm (I group). The length of anal canal

**Table 1.** Morphometrical parametres of rectum

| Object | Anorectal angle, °C | Length of anal canal, mm | Length of pelvic part of rectum, mm | Rectosigmoid Angle, °C |
|---|---|---|---|---|
| Adult human, female | 148±2 | 43±3 | 130±3 | 131±0,7 |
| Adult human, male | 140±3 | 44±3 | 133±2 | 130±0,4 |
| Pig, male | 155±2 | 38±2 | 170±5 | 140±1 |

of males of I group is equal to 43±3 mm and 42 ±3 mm famales of I group. Pelvic part of rectum of pig (II group) is equal to 38±2 mm. The total length of rectum is equal to 173±3 mm (females of I group), 177±5 mm (males of I group), and 208±7 mm (II group) (Fig.1, Fig.2).

The results coincide with the data of literature [16]. Thus, the length of the human`s rectum according to different authors is equal to 12-20 cm, which depends on the location of thepoint of measurements. If the upper limit of the measurement point is the promontorium of the sacral bone, than the length of rectum is 20 cm. If the scientists were guided by the second sacral vertebrate, the length is 18 cm [17]. The proctologists considered [insert 1-2 sources L-ri], that more practicaly to divide the rectum into five departments and to take as initial point of measurement the anocutaneus line: rectosigmoid part (15 to 18 cm), upper ampular division (12 to 15 cm), middle ampular division (7 to 12 cm from the anus), the lower ampular division (up to 6 cm from the anus), and the perineum department or anal canal (length from 1.5 to 4 cm) [18-20].

As a result of anatomist`s researches, it was determined that the length of the anatomical anal canal is about 2 cm (distance from the rectocutaneal line to the pectineal line. Surgical anal canal includes not only the anatomical anal canal (the upper border is anorectal line), but the distal part of the rectum to the upper edge of the muscle ring (internal and external anal sphincters), which is clearly defined by the proctologist during the finger examination. The difference in identification between clinical and anatomists term can be explained with features of blood supply, inertia, venous and lymph nodes [21, 22, 23].

The diameter varies depending on the part, the diameter of the supraampular part is 4 cm, the ampula -7 cm, the diameter of the anus – 3 – 6 cm and it looks as fissure, the appearance of a slit at the level of the anus. This result is coincided with the data of literature.

In the foreign modern editions the term «anorectum» is used and its diseases are described as a number of non-tumor diseases, such as anococcycoccal pain syndrome, proctalgia, coccigodynia, characterized with pain sindrom accompanied with the presence of pain (sigle clinical manifestation, especialy at night) in the area of anus [24, 25, 26]. The anus can be located deep, to be conus-shaped in case of good developed peroneal muscles, or to be plane in case of prolate peroneal muscles (after delivery, ageing, etc.).

Via 3D reconstruction is defined that anorectal angle of rectum is equal to 140-150 °C, thus anorectal angle of rectum of males of I group is equal to 140 ± 3 °C, and anorectal angle of females of I group is equal to 148 ±2 °C. This difference is size can be explained with age changes of peroneal muscle tonus and delivery (femail`s perineum). At same time the location of anorectal angle was determined: in case of 150 °C rate, the anorectal angle was located below pubococcigeal line. Ar same time, the length and volume of anal canal was in norm, overwise it coud be reason of sphincter apparatus disorders. Not a single person from I group did not complain of encopresis or incontinence symptoms.

The anorectal angle of pig`s rectum (I experiment group) is equal 152±2 °C, that according to us is explained of animal posture (tetrapod type).

## CONCLUSIONS

Due to the 3D reconstruction of the pig`s and human`s rectum structure, the morphologic similarity in the form and sizes is established. On the basis of this data we conclude that pigs can be used as a model for experimental and clinical researches in order to develop the newest methods for treatment of rectal pathology, including the development of surgical intervention methods.

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ORIGINAL ARTICLE

# ACTUAL PROBLEMS OF DERMATOVENEROLOGICAL SERVICE DEVELOPMENT: OPINION OF SERVICE PROVIDERS

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## ABSTRACT

**The aim:** To determine the features and trends of dermatovenerological health of population; to identify conditions and problems of providing dermatovenerological care.

**Materials and methods:** Bibliographic, epidemiological, medico-statistical, sociological methods were used.

Scientific literature, data from the Center of Medical Statistics of the Ministry of Health of Ukraine concerning population health and dermatovenerological establishments activity on 2000-2019, data of authors` own sociological researches on opinion of dermatovenerological profile experts about available problems and ways of their decision were analyzed.

**Results:** A long-term tendency towards a decrease in the incidence of skin and subcutaneous tissue diseases by 8,6% during 2000-2017 and the incidence of venereal diseases during 2000-2019 was revealed (for syphilis – by 15,5 times, gonorrhea – by 6,8 times). At the same time, during 2014-2019 there was an increase in the incidence of atopic dermatitis by 3,6%, of contact dermatitis – by 5,2%, of trichophytia and microsporia by 22,6%, as well as increase in the prevalence of psoriasis – by 6,0%. The incidence of sexually transmitted diseases remains significant. Trends in recent years indicate a deterioration in dermatological health and an increasing need for medical services. During healthcare reforming over the course of nineteen years there was a reduction in the amount of dermatovenerologists and their number per population by 31,5%, the amount of dermatological institutions – by 2,6 times and beds – by 3,9 times. According to 18,6±3,1 per 100 surveyed respondents, the availability of dermatovenerological care to the population is low, and its deterioration in the COVID-19 pandemic was confirmed by 80,1±4,0. Some factors that negatively affect the quality of medical services have been identified: insufficient amount and quality of medical equipment (57,1±4,0 per 100 respondents), insufficient opportunity for continuous professional development (34,0±3,8); lack of computers (30,8±3,7); lack of motivation to improve the quality of work (28,8±3,6). The priority measures that need to be taken to improve dermatovenerological medical services have been identified.

**Conclusions:** The study of dermatovenerological profile experts` opinion regarding the current state of the dermatovenerological service, human resources, the availability and quality of the services made it possible to identify a number of organizational and administrative problems as well as to prioritize activities on providing dermatovenerological care to the population in the context of healthcare reform and COVID-19 pandemic.

**KEY WORDS:** incidence of skin diseases and sexually transmitted diseases, amount of doctors and beds of dermatovenerological profile, sociological survey, availability and quality of care, COVID-19 pandemic

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## INTRODUCTION

In the context of the national healthcare system reform various healthcare services face a lot of regulatory, organizational, administrative, financial, economic, scientific and educational issues. This issues are associated with trends in population health, changes in the need for various types and profiles of medical services, with implementing new organizational forms of providing services, financing, management, resource generation, with a change in the philosophy and paradigm of healthcare in general.

Achieving the goals of 2030 Agenda for Sustainable Development requires ensuring a proper level of health and well-being of the population, which, in its turn, requires the adaptation of the healthcare system in accordance with challenges and threats of the 21st century. [1].

Particularly noteworthy are four priority areas: population groups and individuals; service delivery processes; promoting health and change management [2].

Current demographic trends indicate significant aging rate, continuous increase in the proportion of older people in the general population age structure, an increase in the proportion of older age groups that require different medical care and support [3].

At the same time, advances in medical technologies make it possible to significantly increase the volume of services provided, coverage of the population by them, as well as to improve the efficiency of healthcare, its availability and quality [4-5].

Considering mentioned above, it is important to ensure the coverage by medical services for all age groups of the population, to improve health indicators and its determinants during a person's life, to reorient healthcare models to an optimal set of population interventions with a focus on the needs of people in individual services [6-7].

A new challenge for healthcare systems is a growing demand for healthcare services amid the spread of COVID-19. Healthcare systems are often overloaded, availability of necessary medical services is reduced and mortality is increased because of disease outbreaks and because of diseases that require prevention and treatment [8].

The ability of any system to continuously provide essential healthcare services depends on the underlying disease burden and the potential of a healthcare system as the

pandemic evolves. Investments in primary medical care are critical in this respect [9].

Healthcare systems faced the challenge of balancing needs related to an immediate response to the COVID-19 pandemic with needs to continuously deliver other essential healthcare services. First of all, this related to providing a safe and effective flow of patients, improving the organization of COVID-19 screening, and targeting patients along appropriate routes. Effective administrative and coordination mechanisms as well as protocols for prioritizing and adapting services help to reduce the risk of healthcare system congestion.

Among the priority issues of providing medical services for the population, attention is needed to the problems of staffing, training and continuous professional development of personnel, as well as to the implementation of a multidisciplinary approach into the process of providing medical services, ensuring accessibility and quality [10-11].

In the list of existing problems of healthcare, and particularly medical care, a special place is occupied by the problems of the development of specialized medical services, one of which is a dermatovenerological service. Its activities are aimed at prevention and qualitative treatment of some socially dangerous and socially significant diseases.

Although the world has made progress in reducing the prevalence of sexually transmitted diseases, about 1 million people are still infected every day. According to the WHO, 376 million new cases of chlamydiasis, gonorrhea, syphilis, trichomoniasis are registered annually, 500 million – of genital herpes, 290 million – of human papillomavirus. These diseases increase the risk of HIV infection, have negative consequences for reproductive health, including infertility, mother-to-child transmission etc. [12].

Improving the provision of dermatovenerological care to the population is extremely relevant for Ukraine, regarding the epidemiological context, a significant need for services, existing financing problems and reorganization changes in the national healthcare system. The urgency of this issue increases due to the implementation of the Association Agreement between Ukraine and the European Union.

Research of features and trends of dermatological health showed that this problem remains relevant and requires the search for new approaches to its solution. [13-16]. In this context, it is important to assess the current state and efficiency of the service, to study foreign experience of its functioning, to identify problematic issues, to clarify the opinions of all participants of the process of providing care dermatological services, as well as to comprehend interdisciplinary approaches and intersectional interaction in the prevention of dermatovenerological pathology, taking into account the possibilities of new technologies and remote forms of service.

In Ukraine there is a lack of research regarding comprehensive solution of the problems of organizing dermatovenerological care in the context of healthcare reform that take into account international standards and approaches, what determined the need for this scientific research and the direction of scientific research.

## THE AIM

To determine the features and trends of dermatovenerological health of population; to identify conditions and problems of providing dermatovenerological care.

## MATERIALS AND METHODS

The methodological basis of the study is based on a systematic approach. Bibliographic, epidemiological, medico-statistical, sociological methods were used.

The study was carried out as part of the research work of Bogomolets National Medical University on the topic "Medical and social substantiation of the optimization of the healthcare organization in the context of the public healthcare system development" (state registration number 0120U100807).

As a scientific base was used Ukrainian and foreign scientific literature on the research topic, data from the Center of Medical Statistics of the Ministry of Health of Ukraine concerning population health and dermatovenerological establishments activity on 2000-2019, data of authors` own sociological researches on opinion of dermatovenerological profile experts about available problems and ways of their decision. The sociological survey covered 156 specialists, 26,3% of which were men, 73,7% were women. The sample included doctors of different ages: 26-35 years old – 25,6%; 36-45 years – 26,9%, 46-55 years – 23,1%; 56-65 years – 16,0%. Most of respondents had more than 10 years of experience, only 16,7% had experience of up to 5 years, 13,5% – from 5 to 9 years.

The tasks of this study are to analyze the state and trends in the incidence and prevalence of dermatovenerological pathology among the population of Ukraine; to assess the scope and results of treatment and prevention activities in healthcare institutions as well as their quality; to determine the problems of professional activities implementation; to identify the problems of staffing in healthcare system and problems of training in the context of reforming the industry.

The object of the study is dermatovenerological health of population, the organization of dermatovenerological medical care.

The subject of the study is the prevalence of dermatovenerological pathology, the scope of activities of the dermatovenerological service, the opinion of dermatovenerological profile experts, the need for resources and organizational support.

Statistical processing and mathematical analysis of research materials were performed by using the program "EXCEL" and modern methods of statistical analysis.

## RESULTS

Population health is the basis for determining the needs for various services, including such important as medical services. In fact, the morbidity in the population as a component of general health characteristic forms the need for outpatient and inpatient treatment, for health-improving and rehabilitation measures.

Analysis of the dermatovenerological morbidity of Ukrainian population revealed many problems that require the improvement of existing system of dermatovenerological medical care organization.

An assessment of dermatological morbidity showed that during 2000-2017 the prevalence of skin and subcutaneous tissue diseases decreased from 4763,9 cases to 4529,1 cases per 100 000 population, or by 4,9%. In the general structure of the prevalence of diseases in 2000, dermatological diseases occupied 3,2% and were at 10th place by rank; in 2017 – 2,6% and 9th place by rank, respectively.

The incidence of skin and subcutaneous tissue diseases over the mentioned period decreased from 4036,1 cases per 100 000 to 3688,4 cases per 100 000, or by 8,6%. In 2000, as well as in 2017, specific weight of dermatological pathology in the structure of morbidity in the population was 5,9%, which indicates its significant contribution to the formation of disease burden.

In 2017 in the structure of prevalence of skin and subcutaneous tissue diseases the leading places were occupied by skin and subcutaneous tissue infections (24,3%), contact dermatitis (16,4%) and atopic dermatitis (4,6%). Specific weight of these nosologies in the morbidity structure was 28,2%, 18,8% and 2,4% respectively.

Analysis of the morbidity dynamics during 2014-2019 revealed multidirectional trends for different nosological forms. Thus, over a five-year period, the incidence of atopic dermatitis in Ukraine increased by 3,6% and its prevalence increased by 6,6%, the incidence and prevalence of contact dermatitis increased by 5,2% and 5,2% respectively, the incidence of trichophytia and microsporia also increased by 22,6%, the prevalence of psoriasis increased by 6,0%. At the same time, the prevalence of feet mycoses decreased over a five-year period by 3,1%, scabies – by 31,5% (fig. 1).

Preventive examination reveals only about a quarter of all cases of trichophytia (23,3% in 2019), scabies (27,9%) in the population. As for the sexually transmitted diseases morbidity, during 2000-2019 there was a clear tendency towards its decrease. Thus, the incidence of syphilis in the population of Ukraine decreased by 15,5 times, gonorrhea – by 6,8 times (fig.2).

At the same time, its level remains high. Analysis of gender and age characteristics of sexually transmitted diseases in 2019 revealed higher levels of morbidity of men with syphilis by 1,5 times, gonorrhea by 2,0 times compared to women. Among some age groups, the highest incidence of syphilis was in men and women in the age of 35-39 years, very high – in the age of 40-59 years and 20-29 years. The incidence of gonorrhea, chlamydial lymphogranulomatosis, trichomoniasis and urogenital mycoplasmosis in men and women was highest at a young age of 20-24 years.

The prevalence of syphilis according to the contingent of patients who were under dispensary supervision in 2019 was about the same in rural (45,1 cases per 100 000 population) and urban (45,4 cases per 100 000 population) areas. However, the prevalence of gonococcal infection according to the contingent of patients who were under dispensary supervision was three times higher in urban areas.

Preventive examination reveals about two thirds of all cases of syphilis (70% in 2019), about half of all cases of gonorrhea (46,6%), chlamydial infections (47,3%), trichomoniasis (55,1%) and urogenital toxoplasmosis (47,5%).

It is clear that high or low levels of morbidity do not always reflect the true picture of the incidence of pathology among the population, as it is possible not to detect cases due to low availability of healthcare, lack of specialists in the area, low population activity, poor quality of diagnosis, etc. With regard to sexually transmitted diseases, their incomplete detection is also associated with the development of private practice and violations of such cases registration and epidemiological research of contacts. Therefore, in each case there is a need in an in-depth analysis, that takes into account the availability of healthcare facilities, availability and quality of medical care, analysis of patients' opinions.

The study of the resource supply of the dermatovenerological service revealed a decrease in the number of working dermatovenerologists during 2000-2019 by 1274, and the amount of dermatovenerologists positions per population – by 31,5%. The number of positions of pediatric dermatovenerologists decreased during this period by 47 people, and number of dermatovenerologists per pediatric population – by 47,5%. At the same time, there was a decrease in a number of dermatovenerological institutions and offices by 2,6 times, bed stock – by 3,9 times, including for children – by 2,6 times, dermatovenerological beds per population – by 3,3 times, including for children – by 1,9 times.

Analysis of dermatovenerological beds during 2014-2019 showed an increase in the average number of beds occupancy from 317,4 to 320,4 days, especially for children – from 305,3 to 352,0 days. At the same time, the average duration of treatment in specialized beds for adults decreased from 15,7 to 14,9 days; for children – increased from 15,6 to 16,5 days. High occupancy of children dermatovenerological beds and increased duration of treatment on them may indicate a high need for them and the severity of the pathology.

The sociological survey found that 39,7±3,9 per 100 respondents considered themselves as highly qualified specialists, 11,5±2,6 as specialists of average qualification, 47,4±4,0 consider they could not assess themselves. At the same time, 51,3±4,0 per 100 respondents rated the qualification of Ukrainian specialists as average, 40,4±3,9 – as high, and only 8,3±2,2 – as insufficient.

At the same time, 22,4±3,3 per 100 physicians indicated that they cannot always use the available knowledge, skills and competencies in their work due to the lack of modern equipment and technologies; 5,8±1,9 per 100 respondents cannot do this for other reasons.

In general, more than half of the respondents consider the quality of medical services provided to patients as high (55,8 per 100 respondents), 36,5±3,9 consider they cannot assess it, and only 1,3 per 100 respondents consider their quality to be low.

Regarding the availability of dermatovenerological care to patients, 18,6±3,1 per 100 respondents are considered

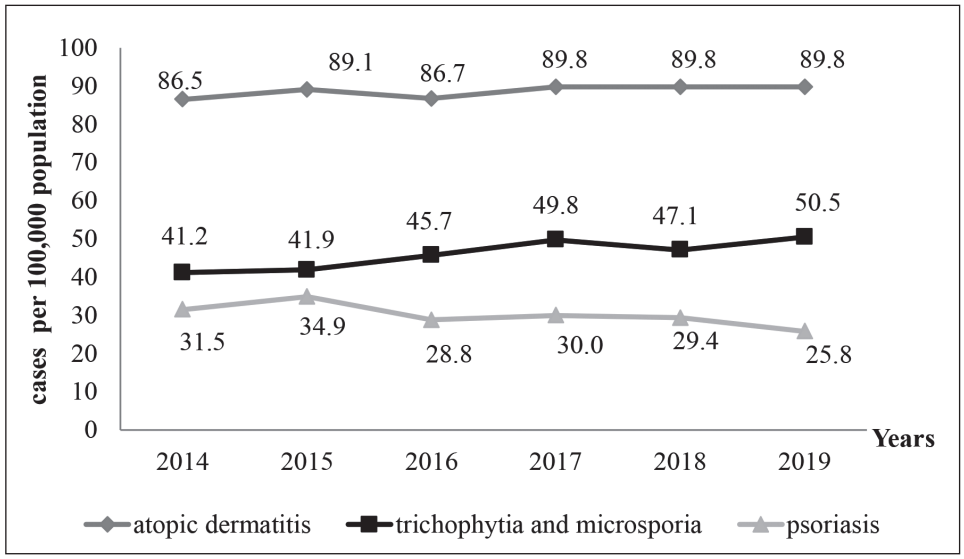


**Fig. 1.** Incidence of atopic dermatitis, trichophytia, microsporia and psoriasis in the Ukrainian population during 2014-2019 (per 100 000)

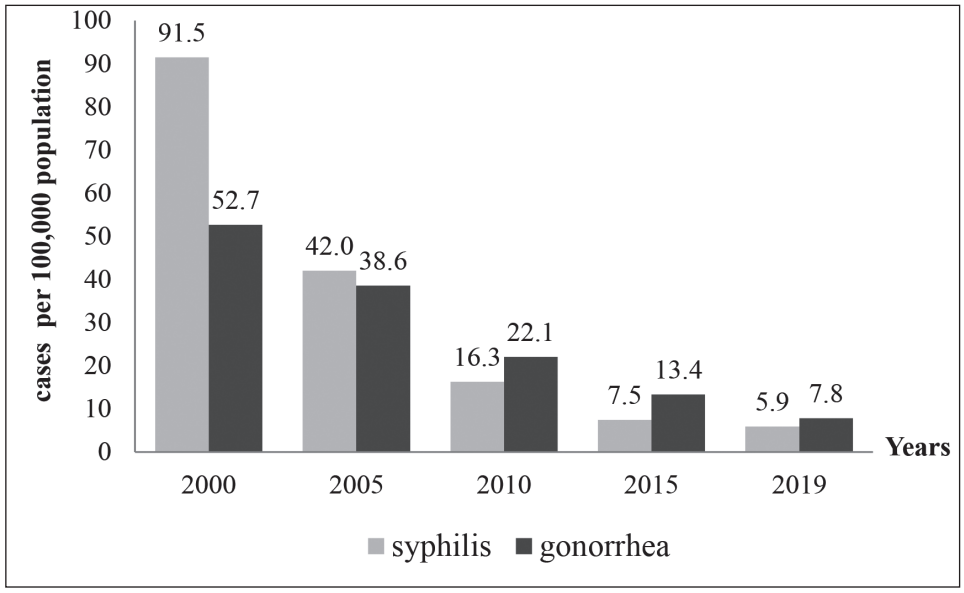


**Fig. 2.** Incidence of syphilis and gonorrhea in the Ukrainian population during 2014-2019 (per 100 000)

the availability as low, 12,2±2,6 cannot estimate; 66,7 ± 3,8 per 100 respondents are considered the availability as high.

Among the reasons that reduce the quality of dermatovenerological care, respondents indicated insufficient medical equipment (57,1±4,0 per 100 respondents), insufficient opportunities for continuous professional education (34,0±3,8); lack of computers (30,8±3,7); lack of motivation to improve the quality of services (28,8±3,6); limited career perspectives (27,6±3,6); insufficient technological equipment (24,4±3,4); unsatisfactory informational support (16,7±3,0); unsatisfactory work organization (14,7±2,8); unsatisfactory coordination of activities with colleagues and support units (11,5±2,6); problems with access to the Internet (9,6±2,4). As it can be seen from the answers, there is a number of resource, technological, organizational and managerial problems in dermatovenerologists working conditions.

The generalized assessment of the resource provision of healthcare facilities in which specialists work showed that it does not always meet the modern requirements – that was indicated by 21,8±3,3 per 100 respondents. The financial support of the structural unit was estimated as mostly sufficient 31,4±3,3 per 100 respondents, mostly insufficient – 36,5±3,9; completely insufficient – 17,3±3,0.

About half of the respondents (46,8±4,0) searched for information with proven efficiency in the sources like Cochrane Library and Medline systems only occasionally, 22,4±3,3 – searched often, while 29,5±3,7 did not perform a search for such information. More than half of the respondents use professional information from foreign sources in their work, but one out of five – not regularly, one out of four – only sometimes.

Assessing the impact of healthcare reform on the volume of their own activities, 10,9 ± 2,5 per 100 respondents noted their increase; 47,4±4,0 – slight increase; 30,1±3,7 did not notice changes.

Dermatovenerologists pointed at the impact of the COVID-19 pandemic on the availability of dermatovenerological care for patients and its quality. Thus, a certain deterioration in the availability of this care for patients was confirmed by 64,7±3,8 per 100 respondents, a real deterio-

ration in accessibility – 15,4±2,9. At the same time, almost half of the respondents (48,1 per 100) are convinced that despite the pandemic the quality of care has not changed.

Among the existing problems of professional activity, experts pointed out the inadequacy between salary and the level of qualification and quality of work (77,6±3,3 per 100 respondents), relevance of changing the job in the future (44,2±4,0) and hesitation about this change (19,2). As the reasons for the possible job change respondents called the unsatisfactory level of the salary (85,9±2,8), low social status (32,1±3,7), lack of stability (42,9±4,0), lack of career opportunities (18,6±3,1).

The main occupational difficulties faced by dermatovenerologists in the context of the COVID-19 pandemic were the lack of personal protective equipment (63,5±33,9 per 100 respondents), gaps in the management of medical and diagnostic process (57,7±4,0 per 100 respondents), lack of communication between different healthcare facilities (56,4±4,0 per 100 respondents) as well as between healthcare units and professionals (51,4±4,0 per 100 respondents), lack of knowledge in the management of patients with coronavirus infection in a pandemic (42,3±4,0 per 100 respondents), bad sanitary and epidemiological activities in healthcare facilities (17,9±3,1).

Experts noted a decrease in medical activity of patients with dermatological diseases – that was indicated by 60,9±3,9 per 100 respondents, and 23,7±3,4 noted a significant decrease, which, in their opinion, led to late treatment and late diagnosis of diseases.

Among the priority measures that need to be taken to improve the medical care of patients with dermatovenerological diseases during the pandemic, 76,9±3,4 per 100 respondents indicated the use of digital platforms for the provision of dermatovenerological services (websites, informing systems, electronic prescriptions, automated programs for tracking the supply and availability of equipment, medicines, etc.), 75,6±3,4 – pointed at providing health care professionals with personal protective equipment. As priority measures respondents have also identified the improvement of healthcare funding while taking into account the growing needs of the pandemic (62,2±3,9), implementing an effective system of staff motivating (46,2±4,0), providing healthcare facilities with the necessary human resources (41,0±3,9), improving the logistics of healthcare facilities (42,9±4,0), improving the technological equipment of healthcare facilities (37,2±3,9), ensuring safety regime for patients and employees in health care facilities (26,5±3,5).

Improving the effectiveness of prevention of dermatovenerological pathology in a pandemic will contribute to its improvement at the level of primary (85,9±2,8 4 per 100 respondents) and specialized (89,1±2,5) medical care, active development of the public health system (41,7±3,9) and improving the provision of collective public health services, for example active informing the population about the risks of dermatovenerological pathology, advocacy, communication, social mobilization in the interests of health (41,7±3,9), effective epidemiological surveillance and monitoring of the situation with COVID-19 and the incidence of dermatovenerological pathology (36,5±3,9), implementation of measures for promoting population health, improving the socio-economic determinants (22,4±3,3), resumption of the sanitary-epidemiological service for effective healthcare, including environmental control (15,4±2,9).

## DISCUSSION

The results of the sociological survey of dermatovenerologists are important basis for substantiating the rationale for measures to improve the organization of medical care. They showed the responsible attitude of specialists to the assessment of their own professional level, to the professional level of their colleagues, to the state of dermatological care, its availability and quality, to the existing problems of prevention and treatment processes as well as the impact of healthcare reform and the COVID-19 pandemic. At the same time, they allowed to identify the priority ways to solve the existing problems (according to dermatovenerologists).

This is especially important to consider taking into account the prevalence of dermatovenerological pathology. After all, the negative trends of growth in the incidence of atypical dermatitis by 3,6%, contact dermatitis by 5,2%, trichophytia and microsporia by 22,6%, as well as growth in the prevalence of psoriasis by 6,0% during 2014-2019 indicate a worsening of dermatological health and increasing need for health services.

Despite the decline in the incidence of sexually transmitted diseases in recent decades, its level remains unacceptably high. Young people in the age of 20-35 need special attention, because the highest incidence of syphilis, gonorrhea, chlamydial lymphogranulomatosis, trichomoniasis and urogenital mycoplasmosis is observed in this age group. Prevention of sexually transmitted diseases should begin at early age to cover all segments of the population because the age of 20-35 is an active fertile age, and there is a high probability of adverse effects of sexually transmitted infections on reproductive health and neonatal health. In the context of defects in the registration of sexually transmitted diseases in the private network of healthcare facilities, it is necessary to improve the accounting and epidemiological investigation of cases.

Given the significant reduction in the positions of dermatovenerologists during the nineteen-year period and their amount per population by 31,5%, the amount of dermatological institutions – by 2,6 times and beds – by 3,9 times, the assessment of the of conditions of dermatovenerological service functioning by physicians reveals existing problems, current realities and outlines perspectives. In this context needs attention the low availability of dermatological care (that was indicated by 18,6±3,1 per 100 respondents), reasons for its quality decline, such as insufficient medical equipment (as indicated by more than half of respondents); lack of computers (confirmed by a third of respondents); low motivation, imperfect technologies, organizational and managerial problems.

Finding effective ways to counter modern challenges and threats requires taking into account such realities as the COVID-19 pandemic, which has reduced the availability of services, reduced the medical activity of patients with dermatovenerological diseases and tightened the conditions for providing the necessary care. Identifying barriers to qualified patient care – which, according to physicians, are associated with a lack of personal protective equipment, administrative errors, lack of communication strategies and lack of knowledge on managing patients with coronavirus infection – identifies areas for improving service delivery, prioritizes them and determines the need in an intersectoral approach to problem solving and development of public health services and its interaction with the medical network.

## CONCLUSIONS

Significant levels and tendencies of increase of dermatological pathology among the Ukrainian population, high rates of sexually transmitted diseases morbidity, especially among young people and people of reproductive age, their negative effects on health provoke the need to improve the dermatovenerological healthcare service.

A significant reduction in the resource supply of the dermatovenerological healthcare services, including institutions, bed capacity, and the amount of human resources led to an intensification of their use and to decreased availability of healthcare services.

The study of dermatovenerological profile experts` opinion regarding the current state of the dermatovenerological service, human resources, the availability and quality of the services made it possible to identify a number of organizational and administrative problems as well as to prioritize activities on providing dermatovenerological care to the population in the context of healthcare reform and the COVID-19 pandemic.

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ORIGINAL ARTICLE

# INSTRUMENTAL MARKERS OF THE RIGHT VENTRICLE FUNCTIONAL CONDITION WITH TISSUE DOPPLER IN CHILDREN WITH TETRALOGY OF FALLOT AFTER SURGICAL CORRECTION AND THEIR CONNECTION WITH VALVULAR FUNCTION OF PULMONARY ARTERY

10.36740/WLek202011108

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## ABSTRACT

**The aim:** To improve efficacy of the right ventricle functional condition evaluation in children with tetralogy of Fallot after surgical correction by estimation of instrumental markers of myocardial dysfunction.

**Materials and methods:** We completely examined 35 children with tetralogy of Fallot after their surgical correction at the age of 3 – 17 years. For all the patients was presented tissue doppler. We evaluated peak myocardial velocities of right ventrical in different phases of the heart cycle (S, E`, A`), tricuspid annular plane systolic excursion (TAPSE), diastolic myocardial velocities ratio (E/E`), peak myocardial velocity during isovolumic contraction (IVV), isovolumic relaxation time (IVRT).

**Results:** All children of the study group had pulmonary insufficiency of different severity with main predominance of mild pulmonary regurgitation (20 patients, 57,14±8,36 %). Children with tetralogy of Fallot after surgical correction were admitted with: decreased TAPSE up to 1,39±0,28 cm, decreased S` up to 8,00±1,90 cm/s, and decreased IVV up to 5,69±0,95 cm/s that is significantly lower results of the healthy children. Severe pulmonary regurgitation usually followed by high chances of the right ventricle systolic dysfunction, exactly with: decresed TAPSE<1,5 cm (OR=0,500; 95% CI 0,323 – 0,775), S`<8,1 cm/s (OR=0,600; 95% CI 0,420 – 0,858) and IVV<5,9 cm/s (OR=0,250; 95% CI 0,117 – 0,534). As well we admitted significant decline of the velocities in earl and end diastole periods to compare with the results of the control group (E`= 12,11±1,22, A`= 4,56±0,92 cm/s (P=0,009 and P=0.0002)), boost of the E/E` ratio – 7,96±2,33 (P=0.01) and decline of the RV IVRT up to 43,49±6,04 ms (P=0.017).

Severe pulmonary regurgitation followed by high chances of the right ventricle systolic dysfunction development with TAPSE <1,5 cm (OR=0,500; 95% CI 0,323 – 0,775), S`<8,1 cm/s (OR=0,600; 95% CI 0,420 – 0,858) and IVV<5,9 cm/s (OR=0,250; 95% CI 0,117 – 0,534). As well we noticed high chances of the E/E`ratio > 6,0 in 1,5 times (95% CI 1,072 – 1,903) and decreased E` <12,2 cm/s (OR=0,200; 95% CI 0,083 – 0,481).

**Conclusions:** Apart of clinical symptoms of the heart failure in children with tetralogy of Fallot after surgical correction markers of the right ventricle myocardial dysfunction are presented by indices of myocardial velocities, received during tissue doppler in different phases of the heart cycle.

**KEY WORDS:** tetralogy of Fallot, children, right ventricle, myocardial dysfunction

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## INTRODUCTION

Dominant position in a structure of congenital abnormalities is settled by congenital heart diseases that are more than 30-34 % among all the hereditary defects. According to 2016 analyze through European countries, as well North and South America, Asia, Russia and Ukraine – all of them occupy the same level around 8-10 children per 1000 newborns. In case of natural currency of the congenital heart disease (CHD) mortality rate can be above 40 %, though up to 70 % of children have no any chance to live up to the end of the first year of life [1,2,3].

One of the most common and complicated CHD is a tetralogy of Fallot (TF) – the most obvious "cyanotic" heart disease, that happens 1 in 3500 newborns, and the second for its frequency that has rate closely 7-10 % through all heart abnormalities [2]. Without surgical correction hemodynamic disorders and chronic hypoxemia lead to death in a short term in infant period and average duration of life of such patients doesn't exceed 12-15 years, the second decade of life will be just in 8 % of the patients [2]. Opposite all the above, majority of children that passed surgical correction of TF, for the long time don't have any clinical symptoms, as well as physical activity the same as in absolutely healthy kids [2]. At the same time residual pathology – as obstruction of the right ventricle outflow and chronic pulmonary regurgitation (PR) lead to progressive cardiomegaly, dilation and dysfunction of the right ventricle (RV). It is important to understand that diastolic dysfunction of the RV has asymptomatic course and in its majority manifests before systolic dysfunction,

**Table I.** Echocardiography indicators of the RV systolic function in children with TF and different stages of pulmonary regurgitation

| RV systolic function indicators | Regurgitation severity | | | |
|---|---|---|---|---|
| | Mild n=20 | Moderate n=10 | Severe n=5 | P value |
| TAPSE, (cm) | 1,52±0,31 | 1,23±0,11 | 1,25±0,15 | P=0.38 |
| S`, (cm/s) | 9,03±1,93 | 6,56±0,56 | 6,76±0,21 | P=0.23 |
| IVV , (cm/s) | 6,28±0,54* | 5,16±0,16** | 4,40±0,29 | P=0.005 |

* – the diffidence is significant between group of the mild and severe PR; ** – P=0.040 – the diffidence is significant between group of the mild and severe PR.

**Table II.** Echocardiography indicators of the RV diastolic function in children with TF and different stages of pulmonary regurgitation

| RV diastolic function indicators | Regurgitation severity | | | |
|---|---|---|---|---|
| | Mild n=20 | Moderate n=10 | Severe n=5 | P value |
| E`, (cm/s) | 12,80±1,06 | 11,41±0,56 | 10,74±0,91 | P=0.25 |
| A`, (cm/s) | 5,03±0,35* | 3,84±1,31 | 4,10±0,19* | P=0.029 |
| E/E` | 6,52±1,29* | 8,28±1,72 | 10,66±1,42* | P=0.042 |
| IVRT, ms | 43,85±5,56 | 40,60±3,89 | 47,80±9,14 | P=0.48 |

* - the diffidence is significant between group of the mild and severe PR.

that is why clinical features by themselves cant be the only markers for heart failure (HF) estimation. That is why active search of the laboratory and instrumental markers that confirm development of myocardial dysfunction on preclinical stage is done.

## THE AIM

The aims of this study were to improve efficacy of the RV functional condition diagnostic in children with TF after surgical correction by estimation of the noninvasive instrumental markers of the myocardial dysfunction.

## MATERIALS AND METHODS

We had examined 35 children with TF after surgical correction at the age diapason between 3 – 17 years. Average age of the patients was 9,60±4,71 years. Al the children of the main group were divided according to the duration of the postoperative catamnesis on – up to 5 years and more than 5 years – 12 (34,28±8,02 %) andi 23 (65,71±8,03 %) children totally. For control group we examined 40 healthy children from 9 months up to 18 years (average age 9,44± 0,71 years).

Echocardiography (Echo-CG) was done for all the children with US-scanner «Phillips HDIIXE» by sensor from 3,5 to 7 MHz. The Echo-CG protocol was regulated according to Recommendations for Quantification Methods During the Performance of a Pediatric Echocardiogram: A Report From the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council 2010 with tissue doppler in impulse regime (TDI). With M-mode we checked tricuspid annular plane systolic excursion – TAPSE. TDI with myocardial velocity estimation was done on lateral part of the tricuspid fibrotic annular. As well we measured peak myocardial systolic velocities (S`), peak diastolic earl (E`) and late (A`) velocities, ratio E/E`, velocity during isovolumic contruction of the RV (IVV), isovolumic relaxation time of the RV (IVRT).

Results were statistically proceeded with computer programs IBMSPSS Statistics, version 12 (20). All data were expressed as mean ±SD. Estimation of differences between average meanings was done by coefficient «t» to Students method, percentage values were detected by Fishers method. Approvement of the differences was counted by standard possibility (p) – p<0,05. For difference between comparative values was evaluated ratio of risks. Assessment of the degree of influence of factor characteristics was evaluated by odds ratio for 95%.

## RESULTS

All the children after surgical correction of TF had residual pathology: pulmonary insufficiency (mild – 57,14 %, moderate – 28,57 %, severe – 14,28 %) with constant PR of mild degree and residual pressure gradient on pulmonary artery was 10,83±4,45 mmHg. Children didn't have any complains during the examination period as well they were not detected with symptoms of HF during clinical examination.

**Results of TDI in different phases of cardiac cycle and TAPSE in children with TF and group of control:**

Results of TDI presented decreased meanings of velocity indices of RV myocardial contractions in children with TF after surgical correction to compare with a group of the healthy children, especially declined TAPSE to 1,39±0,28 cm (P=0,029), that on 35,05 % lower healthy children

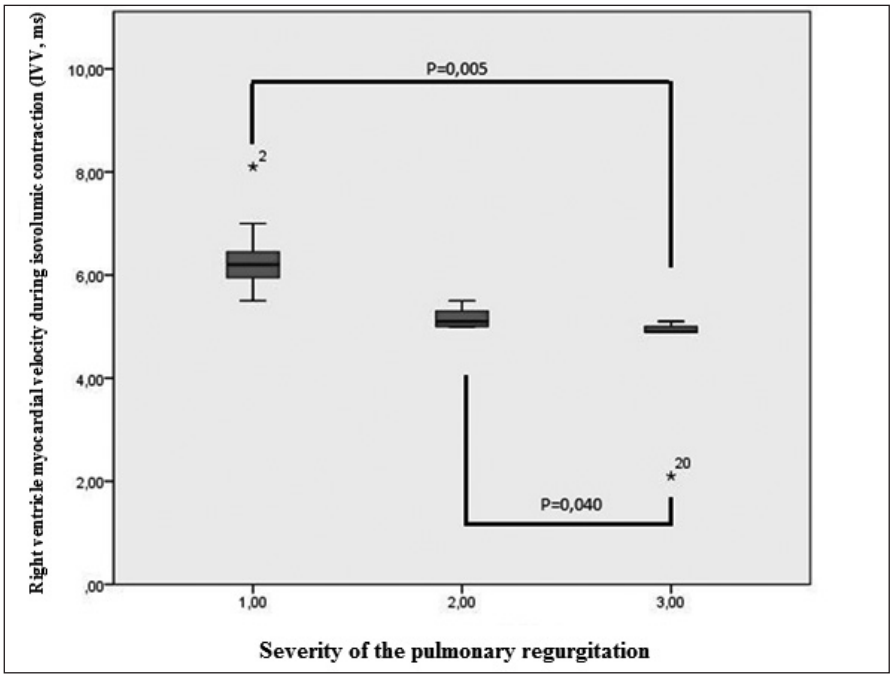


**Fig. 1.** Right ventricle myocardial velocity during isovolumic contraction (IVV) depending on severity of the pulmonary regurgitation.

result, declined S` to 8,00±1,90 cm/s (P=0,031), that lower results of the control group on 37,5 %, declined IVV to 5,69±0,95 cm/s (P=0.034), that on 33,91 % lower the estimated results of the healthy children.

As well we admit significant decline of the velocity indexes in earl and late diastole to compare with control group results (E`= 12,11±1,22, A`= 4,56±0,92 cm/s (P=0,009 and P=0.0002)), increasing ratio E/E` – 7,96±2,33 (P=0.01) and decreased IVRT RV to 43,49±6,04 ms (P=0.017), that indicated worsening of the RV relaxation in patients with TF after surgical correction to compare with healthy children.

**TDI results in different phases of the heart cycle and TAPSE in children with TF according to stage of regurgitation on valve of pulmonary artery:**

While studying correlation between stage of regurgitation on pulmonary artery valve and RV myocardial systolic function, we found out that TAPSE and peak myocardial systolic velocity S` didn't have any significant difference in varying degrees of PR (P=0,38 and P=0,23, accordingly) (tab.1).

At the same time IVV of the RV in case of severe PR was (4,40±0,29 cm/s) and was significantly lower than in case of mild (6,28±0,54 cm/s, P=0,005) and moderate (5,16±0,16 cm/s, P=0,040) (fig. 1).

As well we estimated that more intense PR usually followed by rising of the chance of systolic dysfunction of the RV with decreasing of TAPSE<1,5 cm (OR=0,500; 95% CI 0,323 – 0,775), S`<8,1 cm/s (OR=0,600; 95% CI 0,420 – 0,858) та IVV<5,9 cm/s (OR=0,250; 95% CI 0,117 – 0,534).

Investigation of the relaxation parameters of the RV according to the increase in degree of PR demonstrated that E` and IVRT RV didn't differ significant in three study groups. At the same time result of A` was significantly lower in case of severe PR (4,10±0,19 cm/s), than in variant of mild (5,03±0,35 cm/s, P=0,029) (Tab. 2).

Ratio of diastolic velocities on tricuspid valve demonstrated that progression of PR follows by significant elevation of E/E` ratio (P=0.042) and in severe case was 1,6 times higher than in mild variant (Fig. 2).

We estimated that chance to decrease results of the RV myocardial diastolic function correlates with the stage of PR , especially E` <12,2 cm/s (OR=0,200; 95% CI 0,083 – 0,481), that confirm rising of the unfavorable risk for RV diastolic function in severe variant of PR. As well we noticed increased chance of diastolic velocity ratio E/E` > 6,0 (OR=1,429; 95% CI 1,072 – 1,903), that indicates worsening of the relaxation characteristics of the RV in severe PR.

**TDI results in different phases of the heart cycle and TAPSE in children with TF according to the duration of the postoperative follow-up:**

Peculiarities of the RV myocardial function during systole in children with TF didn't differ in groups before 5 and after 5 years of the patients follow-up (Tab. 3).

Indices of the RV myocardial diastolic function as well were not different in children with period before 5 and after 5 years of the follow-up that indicates earl formation of the relaxation disorders and poor ability of the RV myocardium to compensate residual hemodynamic overloads after surgical correction of the TF (Tab. 4).

Evaluation of the recovery of RV myocardial functionality demonstrated that in postoperative catamnesis over 5 years chance to improve peak diastolic velocity of the tricuspid valve annular lateral part in earl diastole E` rises 2,78 times (OR = 2,783; 95% CI 1,007 – 7,690). Chance to improve other indices of the RV myocardial diastolic function wasn't significant (A` , (cm/s) (OR = 1,18; 95% CI 0,636 – 1,967); E/E` (OR = 1,357; 95% CI 0,635 – 2,899) IVRT, ms (OR = 1,148; 95% CI 0,519 – 2,539)).

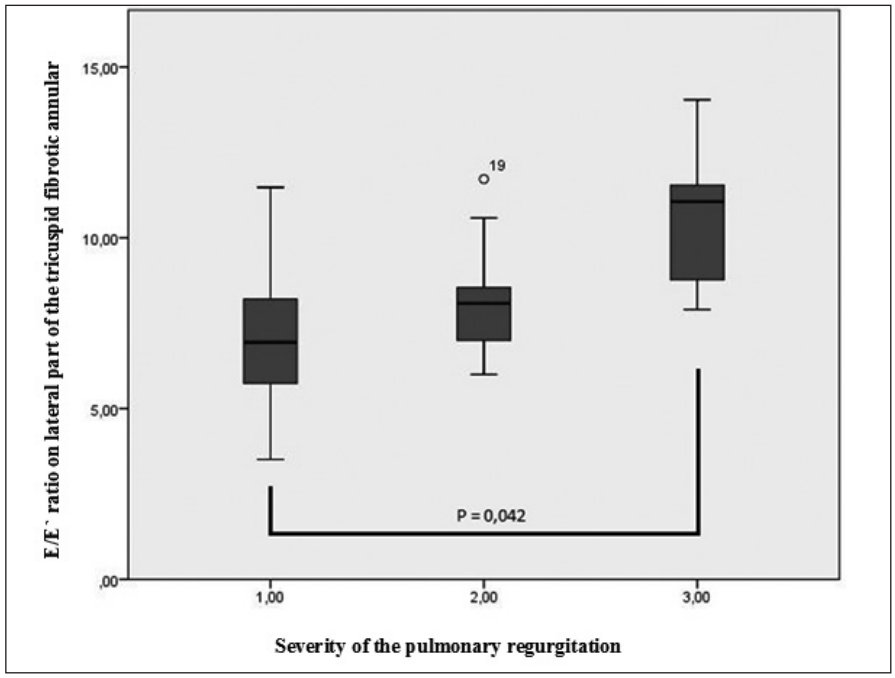


**Fig. 2.** E/E` ratio depending on severity of the pulmonary regurgitation

**Table III.** Echocardiography indicators of the RV systolic function in children with TF according to the duration of the postoperative period

| Indicator | Children with TF, n=35 | | P value | Healthy children, n=40 | P value |
|---|---|---|---|---|---|
| | Before 5 years, n=12 | After 5 years, n=23 | | | |
| TAPSE, (cm) | 1.31±0.23* | 1.44±0.29 | P=0.72 | 2,14±0,19 | P=0.0076 |
| S`, (cm/s) | 7.63±1.80* | 8.19±1.96 | P=0.83 | 12,80±1,09 | P=0.017 |
| IVV , (cm/s) | 5.56±0.52* | 5.76±1.11** | P=0.87 | 8,61±0,99 | P=0.027 |

** P=0.12 – the diffidence is significant between results of the children with follow-up period over 5 years and healthy children.

**Table IV.** Echocardiography indicators of the RV diastolic function in children with TF according to the duration of the postoperative period

| Indicator | Children with TF, n=35 | | P value | Healthy children, n=40 | P value |
|---|---|---|---|---|---|
| | Before 5 years, n=12 | After 5 years n=23 | | | |
| E`, (cm/s) | 11.38±1.02* | 12.48±1.17 | P=0.48 | 16,19±0,91 | P=0.00094 |
| A`, (cm/s) | 4.63±0.53* | 4.52±1.08 | P=0.92 | 9,00±0,71 | P=0.00001 |
| E/E` | 7.67±3.08 | 8.11±1.90* | P=0.90 | 1,81±0,17 | P=0.0016 |
| IVRT, ms | 44.67±5.31* | 42.87±6.40 | P=0.83 | 60,68±3,61 | P=0.016 |

* - the diffidence is significant between results of the children with different duration of the postoperative period.

## DISCUSSION

Children after surgical correction of TF usually have residual pathology that influences on functional condition of RV, that possible may lead to development of myocardial dysfunction. Majority of them after the surgical correction for the long period of time stay assymptomatic that is why active search of the instrumental markers of the HF allows influence on their treatment in time.

Evaluation of the RV in children with TF while using standard echo methods causes several complications due to three anatomical parts places in three squares and can`t be visualized in two-dimensional Echo-CG [4,5]. RV has trabecular structure that complicates estimation of its borders. As well most of the muscle fibres of the RV have longitudinal position that is why determination of its contractility should be done at the same square [6].

TDI – is noninvasive methods that allows estimate longitudinal movement of the RV walls in different phases of the heart cycle and according to the results of myocardial velocities evaluate both systolic and diastolic function. Method is common and easy for calculation with normal reference values available for the pediatric population [7, 8, 9].

By Saxena N and others was estimated that S` of the RV at the level of the lateral part of the tricuspid valve > 10,5 cm/s associated with satisfactory RV function and normal pulmonary artery pressure. But in other studies was

demonstrated that TAPSE< 11.5 cm associated RV EF < 45 % with sensitivity 90 % and specify 85% [10].

In a study of 183 children with TF S`was compared to RV ejection fraction and RV end-diastolic index according to з magnetic resonance imaging (MRI). According to the results of the study S` correlated with RV ejection fraction (r = 0,66, P<0,001) and got negative correlation with S` and RV end diastolic volume index (r = -0,59, P<0,001), that allows to use TDI as alternative method instead of MRI for evaluation of the RV without additional radiation exposure [11]. In our study we checked for the correlation between echo signs of the RV systolic function (TAPSE, S`, IVV) and different stages of the PR. We estimated that TAPSE and S` doesn't have any significant difference in variety of pulmonary regurgitation stages but we found chances for the RV systolic dysfunction in case of more intense PR, lowering of the TAPSE<1,5 cm (OR=0,500; 95% CI 0,323 – 0,775), S`<8,1 cm/s (OR=0,600; 95% CI 0,420 – 0,858) and IVV<5,9 cm/s (OR=0,250; 95% CI 0,117 – 0,534).

E/E` ratio – is an integral sign with the meaning that positively correlates with the pressure of the ventricular filling. In NORRE Study, 2015 p. results of adult population presented for the RV and normal counted E/E` ratio <15 [12]. In our study we estimated correlation between E/E` ratio and stage of the PR. As well we found chance of the increase of E/E` ratio > 6.0 (OR=1,429; 95% CI 1,072 – 1,903), that indicates worsening of the relaxation characteristics of the RV in case of severe PR.

According to the type of the diastolic dysfunction E`, A`, and IVRT can be changed. With results of Radosław Pietrzak and Bożena Werner presented that velocity indices of E` and A`for RV in children with TF after surgical correction were 6,5±2,8 cm/s and 3,8±1,5 cm/s, and were lower than results of the control group (16,2±3,4 cm/s and 7,8±2,0 cm/s) [13]. In a study conducted by Savaş Demirpençe and others reflected similar results during the examination of the children with treated TF (E` – 0,11±0,03 m/s A` – 0,07±0,02 m/s), that is lower than results of the healthy children [14]. In our study we checked velocity indices that characterize relaxation ability of the RV and received similar results (E` – 12,11±1,22 cm/s A` – 4,56±0,92 cm/s), that was lower than results from the control group. Bülent Koca and others in their study compared results received during TDI and heart MRI. Their data shows that velocity indices get through out TDI can be used during follow-up in children with operated TF, but they didn't find any correlation between data received from the heart MRI [15].

Our study shows that some peculiarities of the RV myocardial contractility and relaxation doesn't differ in children before 5 and after 5 years of the follow up that confirm earl formation of the relaxation disorders and poor ability of the RV myocardium to compensate residual hemodynamic overloads after surgical correction of the TF.

## CONCLUSIONS

In a practice of the family doctors, general practitioners, pediatricians , pediatric cardiologists should be counted influence of the residual pathology in case of TF after the surgical correction on functional condition of the RV. Except symptoms of the heart failure, earl indicators of the RV myocardial dysfunction are presented by myocardial velocities during TD in different phases of the heart cycle.

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ORIGINAL ARTICLE

# ASSISTED REPRODUCTIVE TECHNOLOGY EFFICIENCY IMPROVEMENT OF TUBAL-PERITONEAL INFERTILITY

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## ABSTRACT

**The aim:** This study aims to develop and implement a complex of pregravid preparation based on endogenous melatonin level, development of oxidative stress and violation of apoptosis regulation in women with tubal-peritoneal infertility.

**Materials and methods:** We have examined 100 women of reproductive age, including 65 women with tubal-peritoneal infertility treated by ART. All studied women were defined levels of endogenous melatonin, oxidative status and level of apoptosis in the endometrium and in follicular fluid (infertile women only). As a powerful free radical neutralizer, we used melatonin to improve the microenvironment of maturing oocytes and create favourable conditions for implantation in the endometrium.

**Results:** Treatment by exogenous melatonin showed better results than standard therapy without antioxidants: the quality of oocytes and embryos, the level of ongoing pregnancies and childbirth was significantly higher.

**Conclusions:** We achieved such results due to restoring the balance in pro / antioxidant system and apoptosis in female reproductive tract.

**KEY WORDS:** infertility, melatonin, oxidative stress, apoptosis, endometrium, follicular fluid

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## INTRODUCTION

The problem of infertility is an actual question of the world medicine, despite the intensive development of reproductive medicine and embryology. According to the Research by the World Health Organization (WHO) in 2010, 48.5 million couples worldwide were unable to have a child, and about 1 million in Ukraine. In our country, every fifth family is infertile (Kulakov, 2009). In the structure of infertility, the female factor is 30%. Tubal-peritoneal factor is the main cause of female infertility, which reaches 72% of prevalence all forms of reproduction impairment [1] and has no tendency to decrease. Despite is the achievements of modern medicine, the tubal-peritoneal infertility remains one of the most serious pathology, considering the complexity of its diagnosis and treatment, and possibility of restoration of reproductive function.

The most effective way to achieve pregnancy in tubal-peritoneal infertility is use of assisted reproductive technologies (ART). At the same time, the effectiveness of this method does not exceed 40%. The main goal of ART is the birth of a healthy child, so the issue of improving ART and studying new factors that positively influence on implantation with normal pregnancy is the main question of the modern world reproduction. Considerable number of studies are devoted to factors affecting the results of ART [2-8].

Today, the negative influence of oxidative stress (OS) and dysregulation of apoptosis on the reproductive function has been confirmed. According to researches, tubal-peritoneal infertility is characterized by high levels of reactive oxygen species (ROS) in the peritoneal environment, which is responsible for numerous damages of embryo and oocytes [9]. It is known that the adverse effects of ROS include DNA damage, lipid peroxidation and protein damage. Oxidative stress is one of the factors that affect regulation of apoptosis [10,11]. Therefore, development of OS and activation of apoptosis is associated with poor quality oocytes, low fertilization and implantation, a violation of embryos, which significantly reduces the pregnancy rate [12-15]. Thus, the study of OS and the regulation of apoptosis in reproductive tract as potential predictors of ART failures is important.

Currently, interest in antioxidant protection, based on the natural metabolites of cells, is increasing. In this aspect attention is paid to the role of melatonin. It is established that it is effective at system, tissue, cellular and subcellular levels by inhibiting the production of oxygen free radicals and antioxidant protection by activation [16].

Melatonin is not only a regulator of repro and reproduction, but also a powerful antioxidant that controls the antioxidant defence by activating SOD and catalase, binding free radicals of oxygen [17, 18]. Thus, the determination of the level of melatonin, markers of oxidative stress and proapoptotic indices

**Table I.** Demographic, laboratory and anamnestic patient data, characteristics of IVF / ICSI program.

| Groups<br>Characteristics | First group<br>(n=33) | Second group<br>(n=32) | Control group<br>(n=35) |
|---|---|---|---|
| Age*, years | 33,00±1,61 | 33,00±1,21 | 30±1,29 |
| BMI*, kg/m$^2$ | 21,60±1,26 | 23,40±1,39 | 22,9±0,69 |
| Duration of infertility*, years | 6,48±1,68 | 7,03±1,19 | |
| Basal melatonin level**, пг/мл | 20,69±2,44 | 21,72±4,33 | 30,4±1,1 |
| Basal FSH level *, mIU / ml | 7,30 ± 0,63 | 7,11 ± 0,58 | 6,8±0,49 |
| Basal LH level *, mIU / ml | 6,30± 0,98 | 6,02 ± 1,010 | 6,6±0,39 |
| Basal estradiol level *, pg/ml | 60,40±11,17 | 67,70±14,68 | 54,90±4,73 |
| No. of antral follicles * | 15,40 ± 2,45 | 14,10 ± 3,14 | 16,50±1,45 |
| Duration of stimulation *, days | 10,55 ± 0,20 | 10,37 ± 0,30 | |
| Average dose of gonadotropins *, IU | 2059,0 ± 99,7 | 2345,0±89,0 | |
| No. of retrieved oocytes * | 12,80±4,77 | 13,50±5,71 | |
| In vitro fertilisation*, % | 48 | 46,8 | |
| ICSI*, % | 94 | 96,8 | |
| Fertilization rate*, % | 85,7 | 88,4 | |
| No. of transferred embryos * | 1,80±1,32 | 1,08±1,36 | |

* - p <0.05 reliability results of groups relative to each other

** - p = 0.05 reliability results of group 1, 2 relatives to control group

**Table II.** Correlation analysis between the content of antioxidants and the level of f-DNA in follicular fluid and endometrium in group 2.

| Correlation pairs | Correlation coefficient, r |
|---|---|
| SOD in the endometrium - SOD in the follicular fluid | 0,608* |
| Catalase in the endometrium - Catalase in the follicular fluid | 0,698* |
| TBA-products in the endometrium - TBA-products in the follicular fluid | 0,548* |
| APH in the endometrium - APH in the follicular fluid | 0,657* |
| KPH in the endometrium – KPH in the follicular fluid | 0,578* |
| f-DNA in the endometrium - f-DNA in the follicular fluid | 0,538* |

*for a given level of significance p = 0,05, n = 32, critical r = 0.349

in the reproductive tract of women with infertility of tubal peritoneal genesis will allow them to evaluate their fertility, and correction of antioxidant balance in the stage of pregravid preparation will improve the results of ART.

Melatonin is not only a regulator of reproductive maturation and reproduction, but also a powerful antioxidant that controls the antioxidant defence by activating SOD and catalase, binding free radicals of oxygen [17, 18]. Thus, the determination of the level of melatonin, markers of oxidative stress and proapoptotic indices in the reproductive tract of women with tubal-peritoneal infertility will allow to evaluate their fertility, and correction of pro/antioxidant balance in the stage of preparation for pregnancy will improve the results of ART.

## THE AIM

Develop and implement a complex of pregravid preparation based on the level of endogenous melatonin, the development of oxidative stress and the violation of the regulation of apoptosis in women with tubal peritoneal infertility.

## MATERIALS AND METHODS

### STUDY POPULATION

We have examined 100 women of reproductive age, including 65 women with tubal-peritoneal infertility treated by ART in Clinic of reproductive technologies by Ukrainian State Institute of Reproduction, Kiev. They were divided into two groups, depending on the medical preparation for ART (in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI)). The first group included 33 women who received antioxidant therapy "Vita-melatonin" (JSC "Kyiv Vitamin Factory", Ukraine) at a dose of 30 mg inside for 3 months before and during 2 weeks of ovarian stimulation in the IVF program. [19] Approval for this study was obtained from the local Ethics Committee of NMAPE and expressed informed consent was obtained from women before IVF-ET treatment. The second group included 32 women who received treatment according to the traditional regimen of controlled ovarian stimulation (COS) with gonadotrophins.

65 candidates with tubal-peritoneal infertility in reproductive age, with body mass index (BMI) not more than

30 kg / m2, with normal level of reproductive hormones, regular menstrual cycle (25 – 35 days), normal responders with more than 6 antral follicles on ultrasound and FSH less than 10 mIU/ml and normozoospermia were included in study. Exclusion criteria were patients with structure disorders of reproductive system, male and another female factor of infertility. The control group consisted of 35 conditionally healthy women of reproductive age with implemented reproductive function.

## ART

COS by gonadotropins was performed according to the standard long-term protocol with gonadotropin-releasing hormone agonist (a-GnRH). Patients were administered the a-GnRH triptorelin (S.R. liof. do inj. 3,75 mg, Ipsen Pharma) starting in the mid-luteal phase of the preceding cycle. On 2-3 days of menstruation started stimulation with gonadotropins (follytropin alfa or follytropin beta). Doses of gonadotrophins were selected individually, considering the hormonal status, age, and ultrasound monitoring parameters follicular growth and endometrial thickness. As the trigger final oocyte maturation, we used human chorionic gonadotropin (hCG) in ovulatory dose (Horahon, Ferring GmbH, Germany, 10000 units) which were administered an average of 11 days of stimulation in both groups.

Oocyte retrieval was performed by transvaginal aspiration 35-36 hours after hCG injection with needles Luer, 17G (Henke, Germany). The assessment of the quality of oocytes was carried out in terms of maturity: mature – MII (oocytes in the stage of metaphase 2 meiosis) and immature: MI (in metaphase I meiosis), Gv (oocytes at the stage of the germinal vesicle), Atr (atretic oocytes that are not capable of fertilization). The fertility of spermatozoa was evaluated in accordance with WHO 2010 recommendations (WHO., 2010). Fertilization of mature oocytes in vitro or ICSI was carried out in embryological laboratory by biologists according to the standard method. The quality of embryos was estimated at 5th days of cultivation according to Gardner's classification (Gardner et al., 2000. According to this classification, we compiled an integral table by which embryos were divided into 3 types: high-quality, average and low quality (1, 2 and 3). The embryo transfer carried on 5th day of cultivation under transabdominal ultrasound control. 1-2 embryos of high or average quality were transferred.

Luteal phase support was provided by micronized progesterone 600 mg per day vaginally daily, or hydroxyprogesterone caproate 12.5% intramuscularly once every 3 days. To detect implantation, serum ß-subunit of hCG was measured on 12-14 day after embryo transfer. An increase ß-hCG levels greater than 50 mIU / ml was considered a biochemical pregnancy. Absence of ß-hCG increase was classified as fail IVF cycle. Visualization of a fetal egg in uterus by ultrasound in the fifth week was defined as a clinical pregnancy. Loss of pregnancy till 12 weeks was considered as miscarriage. Progressive pregnancy after 12 weeks was defined as ongoing pregnancy.

For a comprehensive assessment of the reproductive system, all patients with infertility were examined according to the Ministry of Health of Ukraine 787 of 09.09.2013 on approval of the use of ART in Ukraine.

## OXIDATIVE STRESS AND APOPTOSIS BIOMARKERS

All studied women were defined levels of endogenous melatonin, oxidative status and level of apoptosis in the endometrium and in follicular fluid (infertile women only). To assess the oxidative status and apoptosis level in the endometrium and the follicular fluid, the prooxidant markers were determined: proteins modification (PM) intensity, thiobarbituric acid (TBA) active products content, and antioxidant markers: superoxide dismutase (SOD) and catalase activity.

Apoptosis level was estimated by content of DNA – fragmentation (f – DNA) in the sample. Endometrial secretion aspiration was in second phase of the menstrual cycle (18-21 days) by catheter. Follicular fluid for research was received during oocyte retrieval after oocyte extraction.

Markers of PM – aldehyde phenylhydrazones (APH) and carboxy phenylhydrazones (CPH) was determined by the method of Dubinina (Dubinina et al., 1995). TBA- active products – by conventional method, which is based on ability of thiobarbituric acid to react with malondialdehyde (Orekhovich, V. N. 1977). SOD activity was determined by Chevari (Chevari, Chaba & Sekei, 1985), activity of catalase – according to the method Korolyuk (Korolyuk, Ivanova, Mayorova & Tokarev, 1988), the content of fragmented DNA was evaluated by the Barton method of diphenylamine test (Barton. K., 1956).

## STATISTICAL ANALYSIS

Statistical data was performed using Microsoft Office Excel program, using the non-parametric T-Wilcoxon T-criteria, and Spirman-based application rank-correlation software. Data are presented as mean (M) ± standard deviation (SD) values. Statistically significant differences and correlation bonds were considered at $p = 0.05$.

# RESULTS

Comparison of three groups by age, BMI, FSH level, LH, oestradiol and number of antral follicles, and two groups by duration of infertility, duration of stimulation, average dose of gonadotropins, number of received oocytes, fertilization rate (IVF / ICSI) and the number of transferred embryos in the cavity of uterus showed no statistical difference ($P > 0.05$). Statistically lower endogenous melatonin level in infertile group in comparison with healthy women group were determined (Tab. I).

The first stage of the study we determined biochemical markers in the studied group of women before the treatment. Comparative analysis showed disbalance between pro- and antioxidants with the trend of oxidative stress and

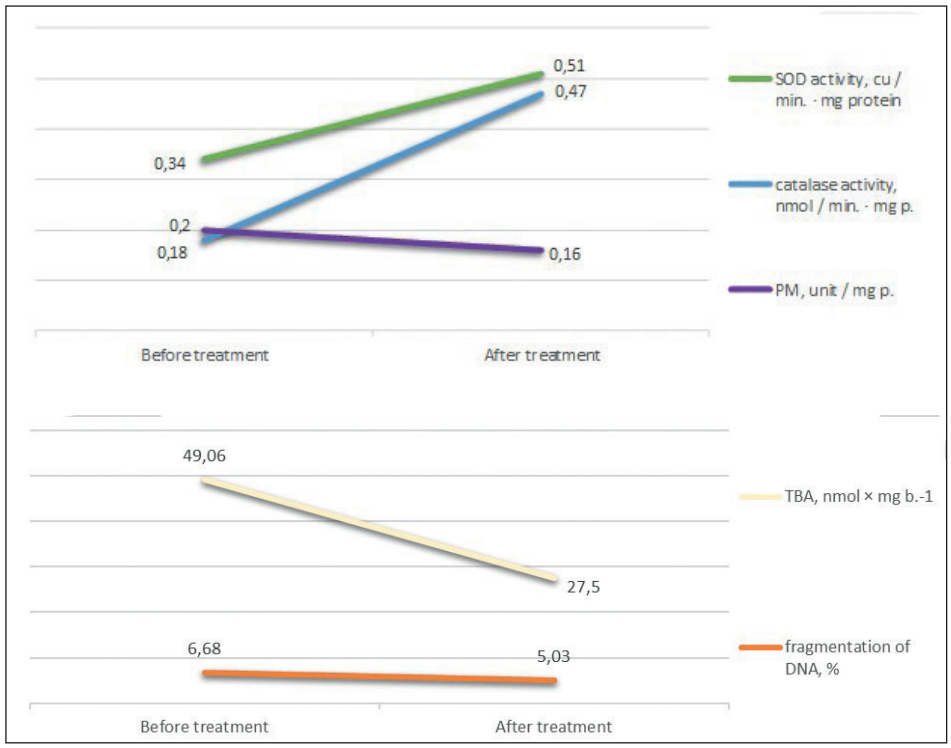


**Fig. 1.** State of antioxidant system and apoptosis activity in endometrium of women from first group before and after receiving antioxidant therapy.

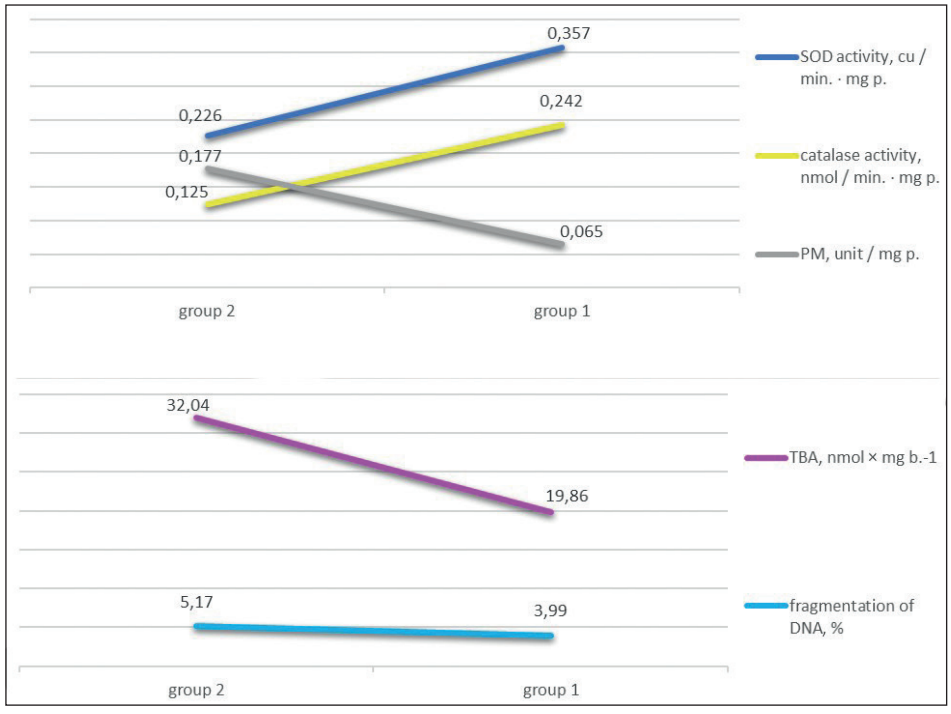


**Fig. 2.** Status pro / antioxidant system and apoptosis activity in follicular fluid of women from 1 and 2 groups.

increased apoptosis in the endometrium of women with tubal-peritoneal infertility as opposed to healthy women [20, 21]. A similar trend was identified in follicular fluid in group of patients who did not receive the antioxidant therapy. We conducted a correlation analysis between pro / antioxidants and f-DNA in the biomaterial of the reproductive tract and found middle direct correlation in the group of women who did not receive treatment with melatonin (Tab. II).

As a result of antioxidant treatment with exogenous melatonin, the level of lipid peroxidation and oxidative modification of proteins decreased by an average of 44% and 24.3%, respectively, while the activity of SOD and catalase increased by 1.5 and 2.6 times respectively; the level of f – DNA decreased by 1.3 times (Fig. 1).

In the follicular fluid of women after antioxidant therapy, there was also a tendency towards a decrease in the level of prooxidants, fragmentated DNA and an increase in anti-

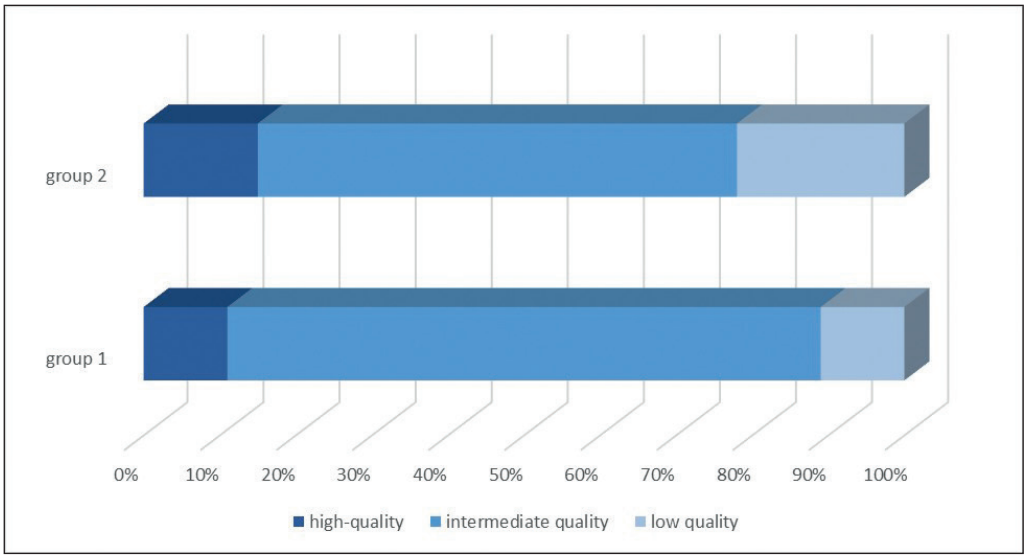


**Fig. 3.** Distribution of embryo quality in study groups.

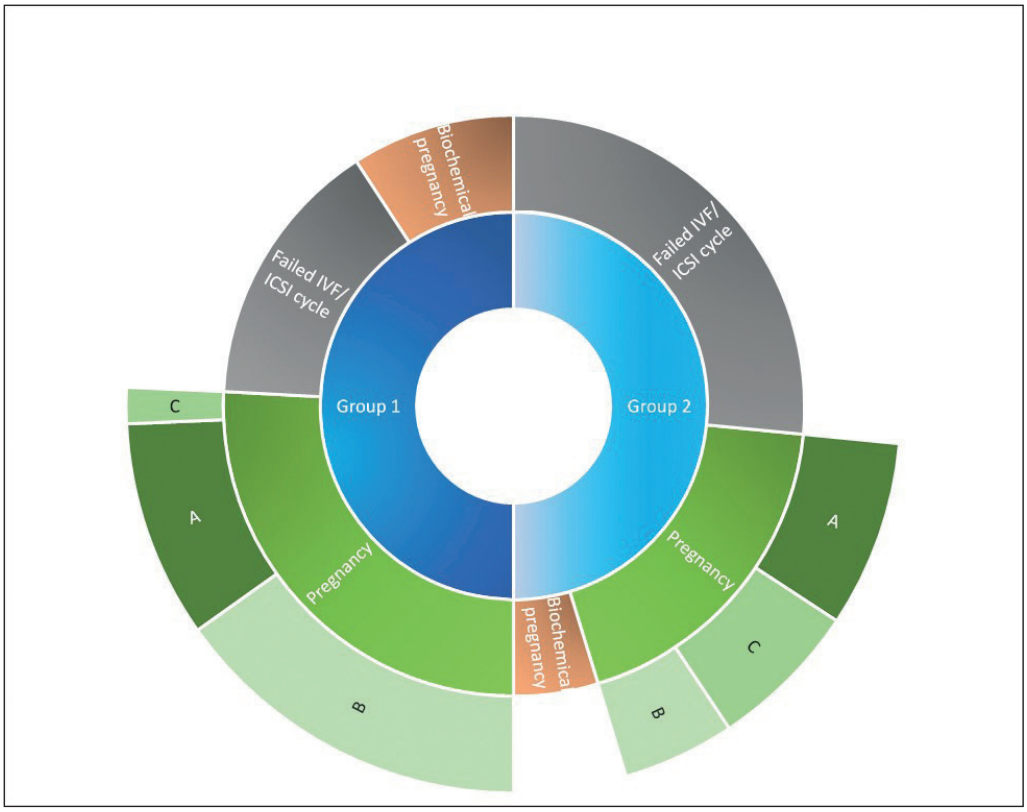


**Fig. 4.** Structure of results IVF program for women with infertility in first and second groups.

oxidant defence: the activity of enzymes SOD and catalase in group 1 was higher in 1.5 and 2 times, compared with the second group, and the levels of prooxidants – lower in 1,7 times. The f-DNA content in the exogenous melatonin group was lower on 23% than in the standard treatment group (Fig. 2). Analysis of these data in both groups showed a significant difference at $p < 0.05$.

Correlation analysis of markers pro / antioxidant system and biochemical indicator of apoptosis showed the relationship between the products of lipid and protein peroxidation and activity of antioxidant enzymes, while the content f-DNA levels correlated only with pro-oxidant. In the follicular fluid, a strong negative correlation was found between catalase activity and f-DNA content ($r = -0.57$) [21].

Evaluation of the quality of oocytes in comparative groups showed a higher percentage of mature oocytes in group 1 relative to the group of women who did not receive the antioxidant: 74% vs. 69%, which is statistically significant. The quality of the oocytes depends on the microenvironment in which they develop, therefore, we analysed the correlation between the prooxidants and the MII oocytes and determined the dependence of number of mature oocytes to a greater extent of protein peroxidation and to a lesser extent of lipid peroxidation and f-DNA in the follicular fluid [22].

Analysis of the embryos of women in both groups showed statistically significant differences: the first group was 25% good quality embryos (1 and 2 quality group) more and in 2 times less embryos of poor quality, but in the second group the number of high-quality embryos in 4% higher ($p \leq 0.05$) (Fig. 3). The higher content of high-quality embryos in the second group was obtained by several women with relatively low content of lipid and protein peroxidation products, DNA fragmentation, high enzyme activity of antioxidant defence and increased levels of endogenous melatonin.

Correlation analysis of high and average quality embryos showed a negative dependence on prooxidants and f-DNA in the endometrium of both groups [22].

Results of ART program infertile women showed a higher percentage of pregnancies in the first group than in the second group – 51.51% vs. 37.51%, respectively. Note the low number of miscarriages in the group that received antioxidant therapy: 3% versus 12.5% in the second group. The level of biochemical pregnancies was 18.18% and 9%, respectively, in groups 1 and 2, which coincides with the average population level (13-22%). Data on the development of pregnancies of the studied groups are presented in Fig. 4.

According to the correlation analysis, pregnancy is associated with the processes of peroxidation of proteins (PM, r = -0,84; r = -0,85) and lipids (TBA, r = -0,71, r = -0,85) in the endometrium and follicular fluid and activation of apoptosis (f-DNA, r = -0,86, p = 0.05) and the activity of enzymes catalase and SOD (r = 0,82; r = 0,84, p = 0, 05) in follicular fluid. It was determined that the pregnancy rate correlates with the number of mature oocytes (MII, r = 0,86, p = 0.05).

## DISCUSSION

Our study showed that the level of TBA-active products and PM markers in endometrium and follicular fluid of women with tubal-peritoneal infertility are elevated and the activity of antioxidant defence enzymes is not sufficient to neutralize free radicals, thus, there is a disturbance in balance between pro- and antioxidants with a tendency to develop oxidative stress. The imbalance in pro / antioxidant system accompanied by increased DNA fragmentation, which also indicates a dysregulation of apoptosis in reproductive tract. Thus, oxidative stress and apoptosis activation in the follicular fluid, which is a microenvironment of maturing oocytes, and in the endometrium, which is a substrate for implantation, can be factors that affect the quality of oocytes, embryos and endometrial susceptibility to these embryos [23]. It was detected an increase of SOD and catalase antioxidants activity and reduction of free radicals under the influence of exogenous melatonin correlate with the successful outcome of ART.

It is believed that in vitro fertilization disturbs the oxidative balance in the culture medium, which makes them less protected from free radicals, and the addition of antioxidants plays an important role in the efficacy of IVF [24]. Some studies confirm the link between development of oxidative stress in the endometrium and female infertility [25]. Consequently, the use of ART in infertility is accompanied by the risk of oxidative stress, so treatment by powerful antioxidants is desirable to provide favourable conditions for in vitro fertilization.

The maturity of the oocytes is associated with a low level of free radicals and enough quantity of unmodified (native) proteins [26]. The imbalance in the system pro / antioxidants in favour of oxidation products and abnormal regulation of apoptosis in the reproductive tract of women is negatively affects the quality of embryos [27]. According to our previous study, we found strong correlation between the quality of oocytes and embryos, that is, a sufficient number of mature oocytes contributes to increase the number of quality embryos [28], which we observed in a group of women who received antioxidant therapy. Thus, the normalization of antioxidant defence contributes improvement of oocyte and embryos quality (due to increase of number of intermediate quality embryos), similar results were obtained by T. Nishihara et al., studying the effect of exogenous melatonin on fertilization rate and quality of blastocyst in infertile women [28].

Pregnancy – is a complex process in which there is a close relationship between embryo and endometrium, it depends on the state of pro / antioxidant system, balance in pro / antiapoptotic factors in the reproductive tract, but the impact of oxidative stress on endometrial receptivity is insufficiently explored [29]. According to the authors [30], the level of SOD in the endometrium secretion was significantly higher in the group with progressive pregnancy, compared with the group with failed IVF cycles. Registration of systemic increase of antioxidants in serum also positively influenced the onset of clinical pregnancy [31]. Revealed correlation dependencies in our study indicate that the normalization of the balance in the system "ROS – antioxidants" promotes pregnancy. Reduction of fragmented DNA is accompanied by a sufficient quality and quality of endometrial cells to ensure its implantation function. So, we found a positive effect of exogenous melatonin on the ratio of pro / antioxidants and apoptosis regulation, as in the endometrium and in follicular fluid, which is closely correlated with the quality of oocytes, embryos, and as a result – improving endometrial implantation ability and pregnancy.

## CONCLUSIONS

Despite the important role of free radicals in the physiological functioning of the reproductive system of women, their excessive content adversely affects fertilization, implantation and pregnancy. Lack of antioxidant enzymes activity also contributes to OS and as a consequence to disruption of apoptosis in the reproductive tract of women with tubal-peritoneal infertility. It was established that correction of this condition by using exogenous melatonin for 3.5 months increases the effectiveness of ART by 14%.

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**Conflict of interest:**
*The Authors declare no conflict of interest.*

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ORIGINAL ARTICLE

# STUDY OF THE STATE OF STRESS-IMPLEMENTING SYSTEMS IN ABDOMINAL DELIVERY DEPENDING ON ANESTHETIC TECHNIQUES

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## ABSTRACT

**The aim:** Evaluation of stress-protective effects of various anesthetic techniques on Cesarean section (CS).

**Materials and methods:** 127 pregnant women who delivered by cesarean section, were divided into 4 subgroups: 1a (n = 31) – general anesthesia (GA) with ketamine, 1b (n = 31) – GA with sodium thiopental, 2a (n = 31) – spinal anesthesia (SA), 2b (n = 34) – SA with intravenous administration of ondansetron at a dose of 8 mg. The assessment was performed at 5 stages: 1 – initial; 2 – infant extraction; 3 – 6 hours after surgery; 4 – 12 hours after surgery.

**Results:** At stage 2, insulin levels in 1a and 1b subgroups decreased by 23.9% and 34.1%, while in 2a and 2b subgroups there were no significant changes. There was an increase in the levels of cortisol, prolactin and cortisol/insulin ratio at the 2 and 3 stages in the 1a and 1b subgroups. Pain intensity increased by the 3 stage in patients of all groups. It was the highest in the 1a and 1b subgroups. At the 4 stage, pain intensity was reduced in all groups, remaining significantly higher in patients of 1a and 1b subgroups.

**Conclusions:** The dynamics of the content of stress hormones, the pain intensity in patients undergoing CS under SA give reason to consider this method as an optimal and adequate one for protection from surgery stress.

**KEY WORDS:** cesarean section, stress hormones, vegetative regulation, general anesthesia, spinal anesthesia

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## INTRODUCTION

Cesarean section (CS) is an indispensable operation that helps to reduce maternal and perinatal infant morbidity and mortality [1]. Selection of anesthetic technique for abdominal delivery is aimed at providing adequate protection of the mother and the fetus from stress reactions caused by surgical trauma [2]. Surgery stress (endocrine, metabolic and inflammatory) response is the most important inducer of dysfunction of various organs and systems (pain, catabolism, pulmonary dysfunction, increased myocardial oxygen demand, gastrointestinal paresis, imbalance and fibrinolysis) [3]. The impact of stress during CS has a negative effect on both the mother and the newborn (post-traumatic stress disorder in women after cesarean section, delayed onset of lactation) [4]. In case of a surgical trauma, somatic impulses from the wound reach the hypothalamus, causing release of hypothalamic releasing hormones, which, in turn, stimulate secretion of the anterior and posterior pituitary hormones [5; 6]. Dynamics of plasma concentration of cortisol, insulin and prolactin is an adequate reflection of the organism response to stress [7-9].

In response to surgery stress, adaptive-compensatory reactions of the organism are formed at the same time, the leading role in formation of which is played by the autonomic nervous system (ANS). The ability to adapt depends largely on the initial state of the ANS. Together with vegetative reactivity and vegetative support, it allows for evaluating homeostatic parameters and adaptive capacity of the organism to stressful conditions [10].

Persons with parasympathicotonic and eutonic type of regulation have a high probability of adequate changes in hemodynamic indices under psycho-emotional stress and physical activity; whereas persons with sympathicotonic type of autonomic regulation have a high probability of excessive stress [11]. Determination of vegetative status helps to define the functional capabilities of the organism and thus to evaluate adaptation reserves.

## THE AIM

The study purpose is to evaluate the stress-protective effects of various anesthetic techniques comprehensively for further development of an algorithm for optimal method of anesthetic technique selection during surgery.

**Table I.** Distribution of patients depending on CS performing time.

| CS | Study groups | | | |
|---|---|---|---|---|
| | 1a (n=31) | 1b (n=31) | 2a (n=31) | 2b (n=34) |
| Planned | 7(22.6±7.5%) | 7(22.6±7.5%) | 21(67.7±8.4%) | 6(17.6±6.5%) |
| Urgent | 24(77.4±7.5%) | 24(77.4±7.5%) | 10(32.3±8.4%) | 28(82.3±6.5%) |

## MATERIALS AND METHODS

The study included 127 pregnant women (average age 29.8 ± 0.5), who were admitted for delivery to public non-commercial utility company "Kharkiv Clinical Maternity Hospital No. 6" from 2015 to 2018. Inclusion criteria: pregnant women aged 18 to 45 with a gestational period of 36-40 weeks. Exclusion criteria: patients in a state of shock of any etiology; decompensated cardiopulmonary pathology; technical failures when performing spinal puncture.

The maternity patients were divided into 2 groups. Group 1 (n = 62) – CS was performed under conditions of multicomponent general intravenous (IV) anesthesia against total myoplegia with artificial lung ventilation (ALV). In this group, the patients were divided into two subgroups depending on the general anesthetic used: 1a (n = 31) – induction (1-2 mg/kg) and maintenance of anesthesia (1 mg/kg) were performed with a 5% ketamine solution; 1b (n = 31) – induction (3-5 mg/kg) and maintenance of anesthesia (1-2 mg/kg) was performed with 1% sodium thiopental solution.

Group 2 (n = 65) – operative delivery was performed under conditions of spinal anesthesia (SA). Spinal puncture (SP) was performed seated at the level L 3-4 with a Quincke needle 25-27 G. Intrathecal 0.5% solution of bupivacaine at a dose of 12-16 mg was added with addition of an adjuvant – 0.01% solution of clonidine at a dose of 0.5 ml. This group of maternity patients was also divided into two subgroups: 2a (n = 31) – SP was performed with central access; 2b (n = 34) – SP was performed with parametric access, and IV ondansetron at a dose of 8 mg was administered 5 minutes before the puncture.

Patients of all groups were premedicated with a 0.1% solution of atropine sulfate at a dose of 0.01 mg/kg. Total myoplegia before tracheal intubation for group 1 maternity patients was provided by IV introduction of a 2% solution of succinylcholine at a dose of 1-2 mg/kg; analgesia was provided by fractional injection of 0.005% solution of fentanyl at a dose of 1-2 μg/kg after infant extraction.

Planned CS was performed in 41 women (32.3 ± 4.1%); 86 (67.7 ± 4.1%) delivered in an urgent manner (Table I).

The degree of operational risk was assessed on the ASA scale. Body mass index (BMI) was calculated by the following formula I.

$$BMI = m/h^2 \quad (I)$$

where m is the body weight, and h is the height in meters.

The pain intensity (PI) was measured at rest and on coughing using a visual analogue scale (VAS).

Hemodynamic monitoring was performed, which included monitoring of heart rhythm and heart rate. Non-invasive measurement of blood pressure indicators – systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) – was also performed. Oxygen saturation ($SpO_2$) was determined by means of patient monitoring using "Leon" apparatus, which meets the requirements of CISPR11 Class A (EN55011, Germany).

In order to determine stress-implementing systems in patients, a complex approach was used, which consisted of determining vegetative status by calculating the vegetative index (VI) by the following formula II.

$$VI = (1- DBP/HR) * 100 \quad (II)$$

where DBP is an indicator of diastolic blood pressure, HR is heart rate per 1 min.

The index value from -15 to +15 indicates total vegetative balance in the cardiovascular system; the value which is more than 15 indicates predominance of sympathetic effects of the autonomic nervous system; the digital value which is less than -15 indicates increased tone of parasympathetic compartment [12; 13].

Study of the level of such hormones as cortisol, insulin and prolactin was performed by means of enzyme immunoassay (Elx-800 enzyme immunoassay photometer, BioTek Instruments, Inc., USA) and a set of reagents for prolactin enzyme immunoassay "Prolaktin-IFA", insulin enzyme immunoassay "Insulin-IFA" and cortisol "Kortizol-IFA" enzyme immunoassay (HEMA Co., Ltd., Russia). Glycaemia level was determined using a biochemical automatic analyzer VITROS 350, Ortho-Clinical Diagnostics, Inc., USA, Liquick Cor-GLUCOSE (PZ CORMAY SA, Poland). The ratio of cortisol/insulin (C/I) was studied: it was calculated by the following formula III**.** Calculation formula C/I is developed by Panin L. E. [14]

$$C/I = \frac{C(at\ study\ stage)*100\%/C(before\ surgery)}{I(at\ study\ stage)*100\%/I(before\ surgery)} \quad (III)$$

where C is plasma concentration level of cortisol, I is plasma concentration level of insulin.

The assessment was carried out in four stages: 1 – initial; 2 – infant extraction; 3 – 6 hours after surgery; 4 – 12 hours after surgery.

Statistical processing of results was performed using the computer program for Windows the SPSS 19 (USA). Quantitative and qualitative variables were used in the statistical analysis. Qualitative data were presented as percentages, and quantitative ones – in the form of mean and standard error (M ± m). The Pearson correlation coefficient was used to determine the relationship between qualitative variables. The critical significance level for testing statistical hypotheses in the study was set at 0.05.

**Table II.** Analysis of clinical anamnesis data of maternity patients in groups.

| Criteria | Group 1 | | Group 2 | |
|---|---|---|---|---|
| | 1a (n=31) | 1b (n=31) | 2a (n=31) | 2b (n=34) |
| Age, years | 28.1 ± 0.9 | 29.1 ± 0.9 | 30.1 ± 0.8 | 31.3 ± 0.9 |
| Gestational age, weeks | 38.1 ± 0.4 | 38.2 ± 0.2 | 38.3 ± 0.2 | 38.3 ± 0.3 |
| Duration of surgery, minutes | 38.9 ± 1.1 | 40.3 ± 1.6 | 38.2 ± 1.6 | 37.5 ± 1.4 |
| Infant extraction duration, minutes | 4.8±0.3 | 4.8±0.3 | 5.5±0.3 | 5.1±0.3 |
| BMI, kg/m$^2$ | 24.2±0.8 | 25.7±1.2 | 24.5±0.7 | 23.8±0.7 |

**Table III.** Types of VNS regulation based on VI at the stages of the study.

| Stage | Vegetative regulation type | Group 1 | | Group 2 | |
|---|---|---|---|---|---|
| | | 1a (n=31) | 1b (n=31) | 2a (n=31) | 2b (n=34) |
| 1 | eutonic | 10 (32.3±8.4%) | 21 (67.7±8.4%) | 17 (54.8±8.9%) | 24 (70.6±7.8%) |
| | sympathicotonic | 18 (58.6±8.9%) | 5 (16.1±6.6%) | 10 (32.3±8.4%) | 9 (26.5±7.6%) |
| | vagotonic | 3 (9.7±5.3%) | 5 (16.1±6.6%) | 4 (12.9±6.0%) | 1 (2.9±2.9%) |
| 2 | eutonic | 5 (16.1±6.6%) | 8 (25.8±7.9%) | 4 (12.9±6.0%) | 10 29.4±7.8%) |
| | sympathicotonic | 26 (83.9%±6.6%) | 22 (70.9±8.1%) | 26 (83.9%±6.6%) | 23 (67.5%±8.0%) |
| | vagotonic | 0 (0.00±0.00%) | 1 (3.2±3.2%) | 1 (3.2±3.2%) | 1 (2.9±2.9%) |
| 3 | eutonic | 11 (35.5±8.6%) | 22 (70.9±8.1%) | 18 (58.6±8.9%) | 23 (67.5±8.0%) |
| | sympathicotonic | 20 (64.5±8.6%) | 6 (19.3±7.1%) | 10 (32.3±8.4%) | 6 (17.6±6.5%) |
| | vagotonic | 0 (0.00±0.00%) | 3 (9.7±5.3%) | 3 (9.7±5.3%) | 5 (14.7±6.1%) |
| 4 | eutonic | 11 (35.5±8.6%) | 22 (64.7±8.2%) | 16 (51.6±8.9%) | 23 (67.5±8.0%) |
| | sympathicotonic | 19 (55.9±8.5%) | 4 (12.9±6.0%) | 10 (32.3±8.4%) | 9 (26.5±7.6%) |
| | vagotonic | 1 (3.2±3.2%) | 5 (16.1±6.6%) | 5 (16.1±6.6%) | 2 (5.9±4.0%) |

## RESULTS

The maternity patients were representative by the main indicators: age, gestational age, duration of surgery and infant extraction (Table II).

The results analysis showed that no statistically significant intergroup differences at initial ANS status were detected among admitted maternity patients ($p > 0.05$). The initial eutonic type of regulation of the autonomic nervous system is predominant. 31 patients (50.0 ± 6.3%) of 1a and 1b subgroups and 41 patients (63.1 ± 6.0%) of 2a and 2b subgroups had the initial eutonic type of regulation of the autonomic nervous system; 23 patients (37.1 ± 6.1%) of 1a and 1b subgroups and 19 patients (29.2 ± 5.6%) of 2a and 2b subgroups had the sympathicotonic type of regulation; 8 patients (12.9 ± 4.3%) of 1a and 1b subgroups and 5 patients (7.7 ± 3.3%) of 2a and 2b subgroups had the vagotonic type of regulation. Most women were characterized by complete autonomic balance and were predicted to develop sufficient adaptive responses to stress, including surgical one. During the study at the stage 2, redistribution of influence of the ANS parts took place. A significant predominance of sympathetic VNS activity was found in patients of all groups, which is an objective indicator and a marker of emotional reactions and presence of stress reaction. 45 patients (72.6 ± 5.7%) had a sympathicotonic type, and 16 patients (25.8 ± 5.6%) had a eutonic type of VNS regulation ($p < 0.05$). In patients undergoing spinal anesthesia,

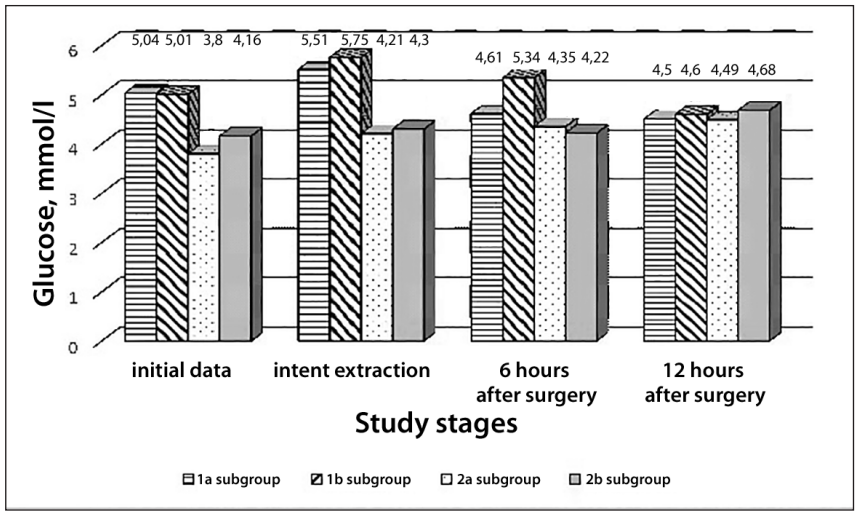


**Fig. 1.** Dynamics of plasma glucose concentration at the main stages of the study.

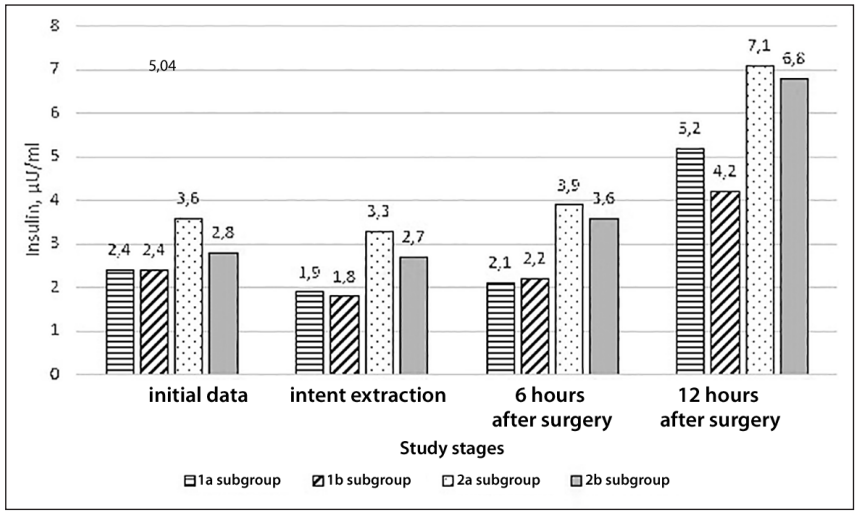


**Fig. 2.** Dynamics of plasma insulin concentration at the stages of the study.

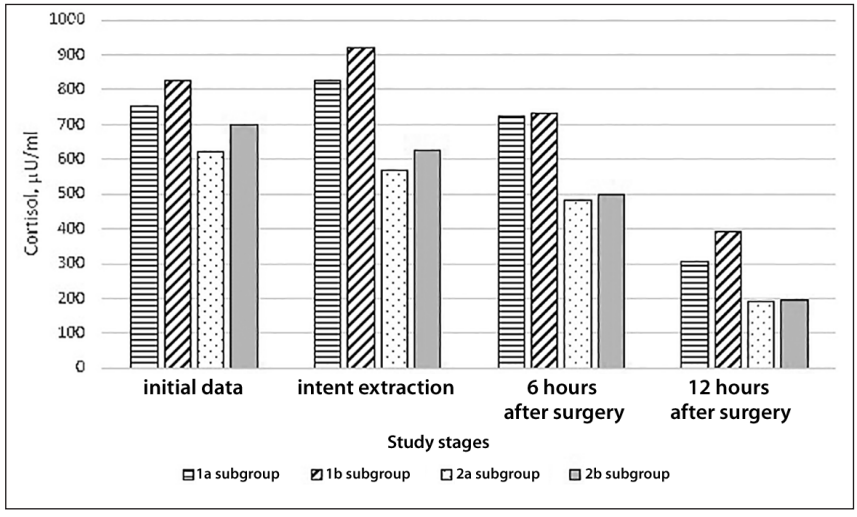


**Fig. 3.** Dynamics of plasma cortisol concentration at the stages of the study.

the autonomic tone at stage 3 returned to the initial level: 41 women (63.1 ± 6.0%) had a eutonic type, and 16 women (24.6 ± 5.3%) had a sympathicotonic type of ANS regulation, whereas sympathicotonia was maintained at all stages of the study in patients of 1a and 1b subgroups, which may indicate a violation of the mechanisms of adaptation to stressful conditions. These patients can be classified to the risk group for development of complications (Table III).

During the study of the hormonal link, there were no statistically significant intergroup differences in initial levels of cortisol, prolactin and insulin. Initial blood glucose levels in patients of 1a and 1b subgroups were significantly

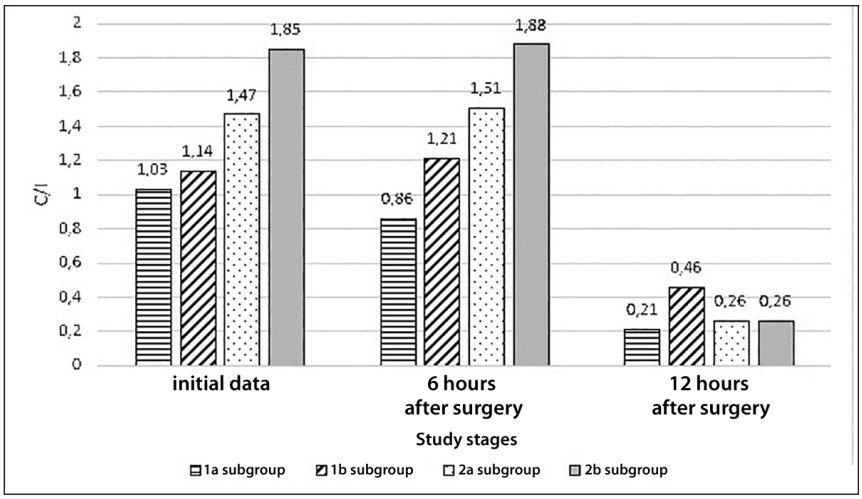


**Fig. 4.** C/I dynamics at the stages of the study.

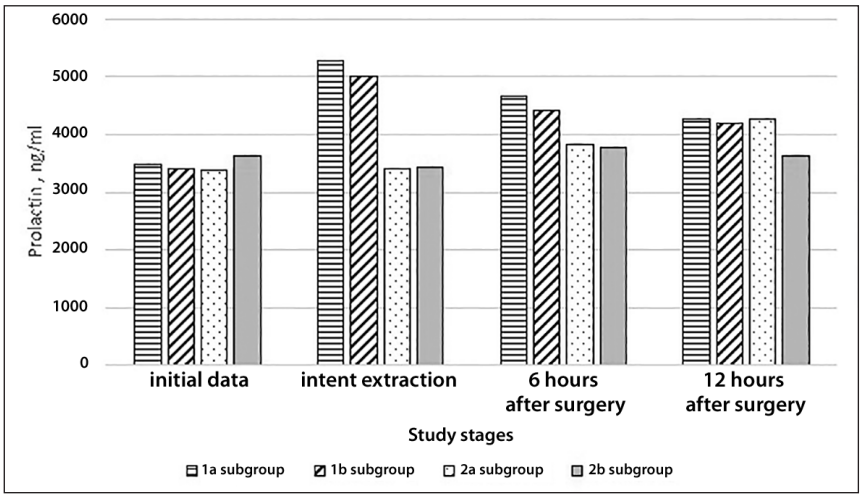


**Fig. 5.** Dynamics of plasma prolactin concentration at the stages of the study.

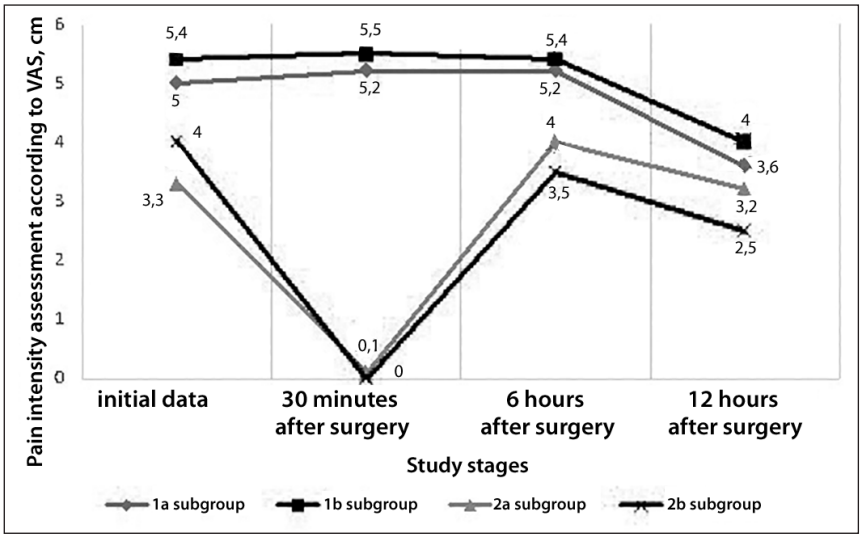


**Fig. 6.** Dynamics of pain intensity et rest according to VAS in study groups at the main stages of the study.

higher (Table III). Hypoglycemia (3.8 ± 0.2 mmol/l) was initially observed in subgroup 2b women. Most likely, this fact is related to a greater number of patients, who underwent planned delivery with the use of enteral nutrition restriction before surgery, in this group (Fig. 1).

At stage 2, blood insulin concentration decreased significantly in women undergoing general anesthesia during cesarean section: in women of subgroup 1a – by 23.9% ($p < 0.05$), in subgroup 1b – by 34.1% ($p < 0.05$) from IL (initial level), whereas in women for whom CS was performed in SA conditions at stage 2, insulin levels approached IL, remaining so without significant variations until stage 3 (Fig. 2). Insulin levels in patients of 1a and 1b subgroups by stage 4 significantly increased twice ($p < 0.001$), but remained significantly

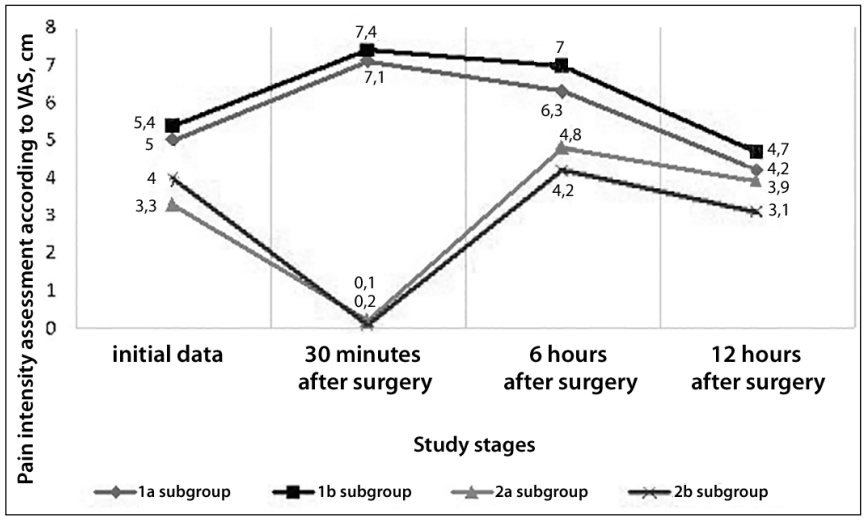


**Fig. 7.** Dynamics of pain intensity on coughing according to VAS at the main stages of the study.

**Table IV.** Indicators of plasma concentrations of stress hormones and glucose levels at the stages of the study

| Stage | Group 1 | | Group 2 | |
|---|---|---|---|---|
| | 1a (n=31) | 1b (n=31) | 2a (n=31) | 2b (n=34) |
| | | Glucose (mmol/l) | | |
| 1 | 5.04±0.2* | 5.01±0.2^ | 3.8±0.2 | 4.16±0.3 |
| 2 | 5.51±0.2* | 5.75±0.2^^ | 4.21±0.1^ | 4.30±0.2** |
| 3 | 4.61±0.2 | 5.34±0.2 | 4.35±0.2^ | 4.22±0.2^^ |
| 4 | 4.12±0.1 | 4.6±0.1 | 4.49±0.1 | 4.68±0.1** |
| | | Insulin (μU/ml) | | |
| 1 | 2.4±0.9 | 2.4±0.3 | 3.6±0.4 | 2.8± 0.3 |
| 2 | 1.9±0.2* | 1.8±0.2 | 3.3±0.3^ | 2.7±0.3 |
| 3 | 2.1±0.2* | 2.2±0.2^^ | 3.9±0.3^ | 3.65±0.2** |
| 4 | 5.2±0.3* | 4.2±0.3^^ | 7.1±0.3^ | 6.8±0.3** |
| | | Cortisol (nmol/l) | | |
| 1 | 753.7±46.9 | 752.6±54.9 | 623.6±60.1 | 699.9±55.6 |
| 2 | 826.3±50.8* | 922.5±52.5 | 569.5±37.04^ | 623.9±42.1^^ |
| 3 | 725.3±39.1* | 732.5±46.2^^ | 481.9±32.4^ | 499.4±29.72** |
| 4 | 305.2±24.3* | 392.03±28.4^^ | 190.9±19.6^ | 194.4±20.3** |
| | | C/I | | |
| 2 | 1.47±0.1 | 1.85±0.2 | 1.14±0.1^ | 1.03±0.9^^ |
| 3 | 1.51±0.3 | 1.88±0.6 | 1.21±0.2 | 0.86±0.2 |
| 4 | 0.21±0.3 | 0.46±0.1 | 0.26±0.1 | 0.26±0.1 |
| | | Prolactin (ng/ml) | | |
| 1 | 3482.1±183.17 | 3402.9±100.2 | 3387.1±155.5 | 3626.1±215.5 |
| 2 | 5271.04±242.7* | 5003.5±193.6^^ | 3411.2±222.9^ | 3443.1±257.6** |
| 3 | 4652.7±168.7 | 4424.3±95.02^^ | 3830.1±202.7 | 3785.7±112.8** |
| 4 | 4272.4±118.6* | 4205.1±140.1 | 4272.1±118.6^ | 3625.3±172.8 |

Notes: * – statistical significance of differences in indicators ($p < 0.001$) between 1a and 2a subgroups;
** – statistical significance of differences in indicators ($p < 0.001$) between 1a and 2b subgroups;
^ – statistical significance of differences in indicators ($p < 0.001$) between 1b and 2a subgroups;
^^ – statistical significance of differences in indicators ($p < 0.001$) between 1b and 2b subgroups.

lower compared to rates in 2a and 2b subgroups (Table III). The concentration of insulin in women who delivered under general anesthesia using sodium thiopental was significantly lower at all stages of the study ($p < 0.001$). During the study of insulin levels, no statistically significant differences were found between subgroups 2a and 2b ($p > 0.05$).

We found an increase in the concentration of cortisol at the 2 stage of the study compared with IL in patients who underwent CS under general anesthesia. In women of 2a and 2b subgroups, the plasma concentration of cortisol decreased slightly at this stage ($p < 0.001$) (Fig. 3). At the 3 stage of the study, a significant difference was also observed: 6 hours after surgery in subgroups 2a and 2b level there was a decrease in cortisol concentration by 29.4% ($p < 0.001$) and by 40.1% ($p < 0.001$), respectively, compared to IL, whereas cortisol levels in subgroups 1a and 1b were close to IL (Table III). At the 4 stage of the study, 12 hours after surgery, the plasma concentration level decreased in all groups, remaining significantly higher in the group of women who delivered under general anesthesia using sodium thiopental ($p < 0.001$). When comparing the plasma concentration of cortisol in patients of 2a and 2b subgroups, no significant statistical difference was found at all stages of the study ($p > 0.05$).

C/I is the main indicator of intensity of adaptation and severity of stress reactions in the organism. The study found high C/I values at stage 2 in women of 1a and 1b subgroups, which remained elevated at stage 3 as well. Patients of 2a and 2b subgroups showed a statistically insignificant increase of C/I ratio at stage 2 compared to initial level ($p > 0.05$) with a decrease of this factor by stage 4 (Fig. 4).

The study of blood prolactin levels revealed a statistically significant increase in the concentration of this hormone at the 2 stage of the study in patients of 1a and 1b subgroups by 51.4% ($p < 0.001$) and 47.0% ($p < 0.001$), respectively, and the increase of prolactin concentration in patients of 2a and 2b groups remained statistically insignificant ($p > 0,05$) (Fig. 5). At the 3 stage of the study, the level of prolactin in the peripheral blood of patients of all groups was determined within the age limits. It shall be noted that it was the highest in women who delivered under general anesthesia (subgroups 1a and 1b) – 4652.7 ± 168.7 ng/ml and 4424.3 ± 95.02 ng/ml, which was significantly different from prolactin concentrations in women who delivered under spinal anesthesia (subgroups 2a and 2b) – 3830.1 ± 202.7 ng/ml and 3785.7 ± 112.8 ng/ml ($p <0.001$). No significant statistical differences ($p >0.05$) were observed between the concentrations of this hormone in patients of 2a and 2b subgroups at all stages of the study (Table III).

There were no statistically significant differences in initial pain intensity in patients in all groups. PI according to VAS in the study groups increased by stage 3 of the study. The PI level at rest in 1a and 1b subgroups was higher – 5.2 ± 0.2 cm and 5.4 ± 0.2 cm ($p < 0.001$; $p < 0.001$) than in patients of 2a and 2b subgroups – 3.9 ± 0.2 cm and 3.5 ± 0.2 cm ($p < 0.001$; $p < 0.001$). By the 4 stage of the study PI was significantly decreased in patients of all groups; significantly higher level of pain at rest was observed in patients of 1a and 1b of subgroups – 3.6 ± 0.1 cm ($p < 0.001$) and 4.0 ± 0.1 cm ($p < 0.001$) compared with 2a and 2b subgroups – 3.2 ± 0.2 cm and 2.5 ± 0.1 cm, respectively ($p < 0.001$; $p < 0.001$) (Fig. 6).

On coughing, the pain was more pronounced compared to the state at rest in patients of both groups. It was the highest in the subgroup 1b patients and was equal to 4.7 ± 0.2 cm, which was significantly different from the pain level according to VAS in 1a subgroup – 4, 2 ± 0.2 cm ($p < 0.001$) and in patients of 2a – 3.9 ± 0.2 cm and 2b – 3.1 ± 0.1 cm ($p < 0.001$) subgroups. Pain on coughing according to VAS in patients in both subgroups of group 2 was lower compared with PI in women in group 1 ($p < 0.001$) (Fig. 7).

In our study, a relationship was found between the qualitative assessment of pain intensity according to VAS and the level of stress hormones. There is a significant correlation between pre-operative VAS PI and pre-operative cortisol and insulin concentrations: VAS – cortisol ($r = 0.68$; $p < 0.001$), VAS – insulin ($r = -0.62$; $p < 0.001$). There is a moderate positive correlation between pre-operative VAS PI and pre-operative glucose and prolactin concentration: VAS – glucose ($r = 0.37$; $p < 0.001$), VAS – prolactin ($r = 0.44$; $p < 0.001$). There is a moderate correlation between VAS PI 6 hours after surgery and cortisol and insulin levels in stage 4: VAS – cortisol ($r = 0.34$; $p < 0.001$), VAS – insulin ($r = -0.38$; $p < 0.001$). There is a weak positive correlation between VAS PI and glucose and prolactin levels 6 hours after surgery: VAS – glucose ($r = 0.260$; $p < 0.05$), VAS – prolactin ($r = 0.264$; $p < 0.05$). There is a weak positive correlation between VAS PI 12 hours after surgery and cortisol and prolactin levels at stage 5: VAS – cortisol ($r = 0.182$; $p < 0.05$), VAS – prolactin ($r = 0.223$; $p < 0.05$), and a negative weak correlation with insulin at stage 5 ($r = -0.274$; $p < 0.05$).

Thus, the higher is VAS PI, the higher are the peripheral blood glucose and cortisol levels are, and the lower is the peripheral blood insulin level.

## DISCUSSION

Vegetative regulation is one of the most important mechanisms of adaptation to stressful conditions. That is why studying the clinical manifestations of predominance of certain parts of the ANS can be an objective indicator of the presence and degree of stress response. In a comparative study of severity of stress reactions when using total intravenous anesthesia during surgery and during the early postoperative period, unidirectional activation of the sympathoadrenal system and the pituitary gland are observed. Cortisol plays the most significant role in the organism response to surgical trauma. The level of plasma cortisol concentration is an adequate reflection of the organism response to surgery stress [3;15-17]. Deficiency of insulin secretion related to stress factors is caused by α-adrenergic inhibition of β-cells secretion with the development of insulin resistance [18]. Deficiency of insulin secretion is combined with catabolic hyperglycemic reaction of the organism. With the extreme influence of various factors that contribute to development of stress, there is an increase of blood prolactin level, and its dynamics largely depends on duration and intensity of this effect [19].

Intra- and postoperative increase of plasma concentration of cortisol, prolactin, cortisol-insulin index, decrease of insulin level in the group of patients operated under conditions of general anesthesia, showed its inability to

protect hypothalamic-adrenal structures and sympathies from intra- and postoperative activation.

During CS under local anesthesia, both afferent entrance from the area of operation to the CNS and hypothalamic-pituitary axis are blocked, as well as efferent vegetative leading pathways to the liver and adrenal glands. Such blockade suppresses adrenocortical and glycemic responses to surgery, as evidenced by lower concentrations of stress-releasing hormones and cortisol-insulin ratio. The use of local anesthesia (compared to total intravenous anesthesia) tends to provide adequate protection, which is more significant, from stress reactions caused by surgery.

## CONCLUSIONS

1. Redistribution of the influence of parts of the ANS in the intra- and postoperative periods in the groups of patients who delivered under general anesthesia is regarded as the organism response to surgery stress. A group of women with initial sympathicotonic type of CNS regulation had initial autonomic tension and cardiovascular load and may be considered at risk for complications.
2. The dynamics of concentration of stress hormones and pain intensity in patients who delivered under spinal anesthesia give reason to consider this method of anesthesia as an optimal and adequate one for protection of patients from surgery stress.
3. The results will allow to develop a mathematical model of selecting the optimal anesthetic technique for each patient individually, considering the initial vegetative tone, which will reduce undesirable and side effects of anesthesia and increase the accelerated recovery of women after cesarean section surgery.

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ORIGINAL ARTICLE

# BAD HABITS AND THEIR IMPACT ON STUDENTS' HEALTH

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## ABSTRACT

**The aim:** Is to investigate the bad habits of the students of higher education institutions and their impact on health.

**Materials and methods:** The research of the students' bad habits was conducted at Polissia National University and Zhytomyr Ivan Franko State University in 2014-2019. 647 students of different specialties between the ages of 17 and 23 were interviewed. The research methods included theoretical methods (the analysis and generalization of literary sources, the evaluation of the quality of students' performance in classes, the study and analysis of the medical records of students); empirical methods (pedagogical observations, questionnaires, surveys); the methods of mathematical statistics.

**Results:** The study found that 32.4 % of male students and 14.9 % of female students smoke. It was defined that male students of the special education department smoke the most – 37.7 %; 32.5 % and 27.1 % of the male students of the main and sports departments smoke respectively. Among female students, 18.4 % of the students of the sports department, 14.6 % – of the main department and 13.4 % – of the special department smoke. The study determined that 2.7 % of both male and female students of the first year of study drink alcohol almost every day, 3.7 % – 2-3 times a week, 18.2 % – once a week, 66.0 % – only on holidays. Besides, the research indicated that some students use drugs. It was found that the students know that smoking, alcohol, and drugs are harmful but cannot or do not want to get rid of these bad habits.

**Conclusions:** The study established that the environment of students is unfavorable for the activation of the healthy lifestyle components; it contributes to the spread of bad habits, the consequences of which students do not realize fully. Many students do not have a need to take care of their own health.

**KEY WORDS:** bad habits, health, physical education, students

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## INTRODUCTION

Taking account of the removal of the discipline "Physical Education" from the list of compulsory disciplines of Ukrainian higher education institutions and reducing the number of academic hours devoted to mastering the program material in this discipline, there is a need to investigate the ways ensuring the efficiency of the students' physical education in accordance with the modern standards of higher education. This becomes especially relevant in a situation of constant deterioration of the psychophysical condition of young students, as evidenced by the results of numerous studies [1, 2, 3]. Conducting various forms of physical education should ensure the engagement of physical culture in the lifestyle of students in order to achieve the optimal level of their physical activity and health. Therefore, many studies have been conducted recently to investigate the problem of improving the content of physical education in extracurricular activities [4, 5, 6]. The need to find innovative approaches to optimizing physical activity to convert the system of physical education into the European model of education, which implies the students' independent choice of the forms, intensity of classes, convenient schedule, etc. At the same time, the higher education institutions (HEI) of Ukraine create insufficient conditions for the formation of students' need to treat their own health as a socio-biological basis of life, as a necessary point for procreation, as a prerequisite of efficient education and creative work, a guarantee of a perfect gene pool, the foundation of the creativity and spirituality development [7, 8, 9].

The problem of preserving the health of young students arises in many countries around the world, which are concerned about the intellectual future of the country. It is developed in various scientific publications [10, 11]. It is conditioned by the fact that over the years of study at HEI, young people do not improve but largely lose the organism reserve capacity that significantly affects their performance. The reason for this is bad habits to a large extent. Because lifestyle directly affects human health, whereas living conditions affect human health indirectly [12, 13].

A student who has health disorders and a poor lifestyle cannot fully realize his or her potential physical and intellectual capabilities [14, 15]. Unfortunately, health does not take the leading position among the needs which are the basis of human behavior, although it should be the main

**Table I.** The students' attitude to smoking (n=647), %

| Attitude to smoking | Gender | The year of study | | | | Total % |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | |
| Do not smoke | males | 63.8 | 57.9 | 54.3 | 55.9 | 59.6 |
| | females | 79.2 | 79.5 | 74.5 | 81.2 | 79.2 |
| | in total | 70.6 | 74.1 | 66.3 | 74.0 | 72.1 |
| Gave up smoking | males | 6.7 | 9.3 | 5.7 | 8.8 | 8.0 |
| | females | 4.9 | 5.8 | 11.8 | 3.5 | 5.9 |
| | in total | 5.9 | 6.7 | 9.3 | 5.0 | 6.6 |
| Smoke: | males | 29.5 | 32.8 | 40.0 | 35.3 | 32.4 |
| | females | 15.9 | 14.7 | 13.7 | 15.3 | 14.9 |
| | in total | 23.5 | 19.2 | 24.4 | 21.0 | 21.3 |
| up to 10 cigarettes a day | males | 12.3 | 20.3 | 20.0 | 20.6 | 16.0 |
| | females | 9.8 | 6.9 | 9.8 | 9.4 | 8.3 |
| | in total | 11.2 | 10.1 | 13.9 | 12.6 | 11.1 |
| up to 15 cigarettes a day | males | 8.6 | 7.8 | 20.0 | 8.8 | 10.5 |
| | females | 4.9 | 7.3 | 3.9 | 4.7 | 5.9 |
| | in total | 7.0 | 7.5 | 10.5 | 5.9 | 7.6 |
| more than 20 cigarettes a day | males | 8.6 | 4.7 | – | 5.9 | 5.9 |
| | females | 1.2 | 0.5 | – | 1.2 | 0.7 |
| | in total | 5.3 | 1.6 | – | 2.5 | 2.6 |

**Table II.** The students' of different educational departments attitude to smoking (n=647), %

| Attitude to smoking | Gender | Educational departments | | |
|---|---|---|---|---|
| | | special | main | sports |
| Do not smoke | males | 49.1 | 60.4 | 67.8 |
| | females | 79.4 | 78.9 | 80.1 |
| | in total | 68.7 | 72.7 | 74.2 |
| Gave up smoking | males | 13.2 | 7.1 | 5.1 |
| | females | 7.2 | 6.5 | 1.5 |
| | in total | 9.3 | 6.7 | 3.2 |
| Smoke: | males | 37.7 | 32.5 | 27.1 |
| | females | 13.4 | 14.6 | 18.4 |
| | in total | 22.0 | 20.6 | 22.6 |
| up to 10 cigarettes a day | males | 22.6 | 15.1 | 11.9 |
| | females | 5.2 | 9.3 | 9.2 |
| | in total | 11.3 | 11.3 | 10.5 |
| up to 15 cigarettes a day | males | 15.1 | 8.7 | 10.1 |
| | females | 8.2 | 4.1 | 9.2 |
| | in total | 10.7 | 5.6 | 9.7 |
| more than 20 cigarettes a day | males | – | 8.7 | 5.1 |
| | females | – | 1.2 | – |
| | in total | – | 3.7 | 2.4 |

need. A healthy lifestyle is a system of human behavior, which is aimed at constant physical improvement, eating culture, getting rid of bad habits (alcohol, smoking, drugs), etc. [16, 17]

Therefore, such social phenomena as drug addiction, alcoholism, unwillingness to live without a sense of life, and its devaluation, non-compliance with all other principles of a healthy lifestyle, and maintaining general health are characteristic of a large number of young students today [18, 19, 20]. The scientific data [21, 22] show that a significant part of students are quite tolerant of alcohol (52.3 %), smoking (34.2 %), drugs (7.7 %), prostitution (28.6 %), which means that many students may already have these habits.

## THE AIM

The aim of the study is to investigate the bad habits of the students of higher education institutions and their impact on health.

## MATERIALS AND METHODS

The investigations of the students' bad habits were conducted at Polissia National University (PNU) and Zhytomyr Ivan Franko State University (ZSU) in 2014-2019. 647 students of different specialties between the ages of 17 and 23 were interviewed. Medical examinations were conducted by the doctors of the medical centers of these higher education institutions.

**Table III.** The students' attitude to smoking depending on the place of residence (n=647), %

| Attitude to smoking | Gender | The place of residence | |
|---|---|---|---|
| | | rural areas (n=341) | urban areas (n=306) |
| Do not smoke | males | 68.5 | 60.2 |
| | females | 84.9 | 74.7 |
| | in total | 78.5 | 69.6 |
| Gave up smoking | males | 9.2 | 10.2 |
| | females | 4.7 | 7.6 |
| | in total | 6.5 | 8.5 |
| Smoke: | males | 22.3 | 29.6 |
| | females | 10.4 | 17.7 |
| | in total | 15.0 | 21.9 |
| up to 10 cigarettes a day | males | 12.3 | 12.0 |
| | females | 6.6 | 8.6 |
| | in total | 8.8 | 9.8 |
| up to 15 cigarettes a day | males | 7.7 | 10.2 |
| | females | 3.3 | 7.1 |
| | in total | 5.0 | 8.2 |
| more than 20 cigarettes a day | males | 2.3 | 7.4 |
| | females | 0.5 | 2.0 |
| | in total | 1.2 | 3.9 |

**Table IV.** The students' attitude to alcohol (n=647), %

| Attitude to alcohol | Gender | The year of study | | | | Total % |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | |
| Drink regularly, almost every day | males | 2.9 | 4.7 | – | 2.9 | 2.9 |
| | females | 2.4 | 1.0 | – | – | 1.0 |
| | in total | 2.7 | 2.0 | – | 0.8 | 1.7 |
| Drink 2-3 times a week | males | 2.9 | 7.8 | 8.6 | 8.8 | 5.9 |
| | females | 1.2 | 3.1 | 2.0 | 2.4 | 2.4 |
| | in total | 2.1 | 4.3 | 4.7 | 4.2 | 3.7 |
| Drink once a week | males | 27.6 | 20.3 | 40.0 | 17.6 | 26.1 |
| | females | 12.2 | 9.9 | 25.5 | 16.5 | 13.7 |
| | in total | 20.9 | 12.5 | 31.4 | 16.8 | 18.2 |
| Drink only on holidays | males | 60.9 | 57.8 | 48.5 | 58.9 | 58.0 |
| | females | 70.7 | 72.4 | 66.6 | 69.3 | 70.7 |
| | in total | 65.2 | 68.7 | 59.2 | 66.4 | 66.0 |
| Do not drink at all | males | 5.7 | 9.4 | 2.9 | 11.8 | 7.1 |
| | females | 13.5 | 13.6 | 5.9 | 11.8 | 12.2 |
| | in total | 9.1 | 12.5 | 4.7 | 11.8 | 10.4 |

The research methods included theoretical methods (the analysis and generalization of literary sources, the evaluation of the quality of students' performance in classes, the study and analysis of the medical records of students); empirical methods (pedagogical observations, questionnaires, surveys); the methods of mathematical statistics.

This study complies with the ethical standards of the Act of Ukraine "On Higher Education" No. 1556-VII dated 01.07.2014 and the Letter from the Ministry of Education and Science of Ukraine "On the Academic Plagiarism Prevention" No. 1/11-8681 dated 15.08.2018. Also, this study followed the regulations of the World Medical Association Declaration of Helsinki – ethical principles for medical research involving human subjects. Informed consent was received from all individuals who took part in this research.

## RESULTS

The research of the students of PNU and ZSU confirmed that 32.4 % of males and 14.9 % of females smoked. In the first year of study, 29.5 % of males smoked; this number increased significantly in the following years and accounted for 40.0 % in the third year. Among females, first-year students smoked the most (Table I). It is a well-known fact that cigarette smoke is harmful not only to smokers but also to everyone around them. Cigarette smoke contains benzene, which is a source of ionizing radiation. Smoking causes lung cancer, is associated with more than 80 % of chronic bronchitis and lung eczema cases, more than 25 % of coronary heart diseases, complicates respiratory diseases, gastric ulcers. Smoking is especially dangerous for pregnant women because it leads to the birth of children with low body weight, physical and mental disabilities.

**Table V.** The students' of different educational departments attitude to alcohol (n=647), %

| Attitude to alcohol | Gender | Educational departments | | |
|---|---|---|---|---|
| | | special | main | sports |
| Drink regularly, almost every day | males | 1.9 | 2.4 | 5.1 |
| | females | 1.0 | 1.2 | – |
| | in total | 1.3 | 1.6 | 2.4 |
| Drink 2-3 times a week | males | 1.9 | 7.9 | 5.1 |
| | females | – | 3.3 | 3.1 |
| | in total | 0.7 | 4.8 | 4.0 |
| Drink once a week | males | 28.3 | 27.0 | 22.0 |
| | females | 18.6 | 11.3 | 15.4 |
| | in total | 22.0 | 16.7 | 18.5 |
| Drink only on holidays | males | 64.1 | 57.9 | 52.5 |
| | females | 67.0 | 72.9 | 67.7 |
| | in total | 66.0 | 67.8 | 60.6 |
| Do not drink at all | males | 3.8 | 4.8 | 15.3 |
| | females | 13.4 | 11.3 | 13.8 |
| | in total | 10.0 | 9.1 | 14.5 |

**Table VI.** The students' attitude to alcohol depending on the place of residence (n=647), %

| Attitude to alcohol | Gender | The place of residence | |
|---|---|---|---|
| | | rural areas (n=341) | urban areas (n=306) |
| Drink regularly, almost every day | males | 0.8 | 4.6 |
| | females | 0.5 | 1.5 |
| | in total | 0.6 | 2.6 |
| Drink 2-3 times a week | males | 4.6 | 9.3 |
| | females | 1.9 | 3.0 |
| | in total | 2.9 | 5.2 |
| Drink once a week | males | 25.4 | 27.8 |
| | females | 11.8 | 16.7 |
| | in total | 17.0 | 20.6 |
| Drink only on holidays | males | 64.6 | 49.1 |
| | females | 74.9 | 65.2 |
| | in total | 71.0 | 59.5 |
| Do not drink at all | males | 4.6 | 9.3 |
| | females | 10.9 | 13.6 |
| | in total | 8.5 | 12.1 |

Among male students, the students of the special education department smoked the most – 37.7 %, followed by the students of the main department – 32.5 % and the sports department – 27.1 %. The females had the opposite trend: 18.4 % of female students of the sports education department, 14.6 % of the main education department, and 13.4 % of the special education department smoked (Table II). A negative example for the students of the sports department is smoking among teachers, coaches, judges, organizers of sports events and the most authoritative sportsmen in teams, there is also a wrong idea that smoking has a positive effect on reducing excess weight.

Another characteristic feature defined was that the students from rural areas smoked less – 15.0 % (22.3 % – males and 10.4 % – females), in comparison with 29.6 % of males and 17.7 % of females from urban areas (Table III).

Alcohol consumption is quite common among students. The study identified that in the first year of study, 2.7 % of students drank alcohol almost every day, 3.7 % – 2-3 times a week, 18.2 % – once a week, 66.0 % – only on holidays (Table IV). Among young students, only 7.1 % of males and 12.2 % of females did not drink alcohol at all.

The research indicated that the work with students concerning the harmful effects of alcohol consumption on sports performance and health was insufficient. The obtained data showed that only 15.3 % of males and 13.8 % of females of the sports departments did not drink alcohol at all. It was found that 2.4 % of the sports department students drank alcohol regularly, 4.0 % – two or three times a week, 18.5 % – once a week (Table V). The students of the sports departments usually have larger areas of communication with their peers; attend training meetings and competitions with no control by parents and teachers, so they follow negative life examples, including smoking, alcohol, drugs, etc. very often.

**Table VII.** The reasons that encourage students to drink alcohol (n=647), %

| The reasons | Gender | The year of study | | | | Total % |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | |
| Resting in company | males | 35.2 | 46.9 | 51.4 | 47.1 | 42.4 |
| | females | 37.8 | 38.7 | 39.2 | 43.5 | 39.6 |
| | in total | 36.4 | 40.8 | 44.2 | 44.5 | 40.6 |
| Meeting with friends | males | 33.3 | 46.9 | 37.1 | 23.5 | 36.1 |
| | females | 34.1 | 32.5 | 31.4 | 27.1 | 31.5 |
| | in total | 33.7 | 36.1 | 33.7 | 26.1 | 33.2 |
| The desire to relax | males | 13.3 | 4.7 | 17.1 | 8.8 | 10.9 |
| | females | 11.0 | 6.8 | 7.8 | 1.2 | 7.8 |
| | in total | 12.3 | 6.3 | 11.6 | 7.6 | 9.0 |
| Boredom | males | 8.6 | 1.6 | 8.6 | 8.8 | 6.7 |
| | females | 6.1 | 2.6 | – | 2.4 | 2.7 |
| | in total | 7.5 | 2.4 | 3.5 | 3.4 | 4.2 |
| Meeting with parents | males | 2.9 | 1.6 | – | 2.9 | 2.1 |
| | females | – | 1.0 | 2.0 | 1.2 | 1.0 |
| | in total | 1.6 | 1.2 | 1.2 | 1.7 | 1.4 |
| Do not have such need | males | 41.0 | 37.5 | 25.7 | 32.4 | 36.6 |
| | females | 40.2 | 40.8 | 43.1 | 41.2 | 41.1 |
| | in total | 40.6 | 40.0 | 36.0 | 38.7 | 39.4 |

**Table VIII.** The subjective assessment of the students' attitudes to drug use (n=647), %

| The subjective assessment | Gender | The year of study | | | | Total % |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | |
| Use drugs | males | 2.9 | – | – | – | 1.3 |
| | females | – | 0.5 | 2.0 | 2.4 | 1.0 |
| | in total | 1.6 | 0.4 | 1.2 | 1.7 | 1.1 |
| Tried drugs | males | 4.8 | 3.1 | 2.9 | 17.6 | 5.9 |
| | females | 1.2 | 1.6 | 7.8 | 2.4 | 2.2 |
| | in total | 3.2 | 2.0 | 5.8 | 6.7 | 3.6 |
| Do not use drugs | males | 92.3 | 96.9 | 97.1 | 82.4 | 92.8 |
| | females | 98.8 | 97.9 | 90.2 | 95.2 | 96.8 |
| | in total | 95.2 | 97.6 | 93.0 | 91.6 | 95.3 |
| Know drug effects | males | 3.8 | 9.4 | 11.4 | 2.9 | 6.3 |
| | females | – | 2.1 | 9.8 | 1.2 | 2.4 |
| | in total | 2.1 | 3.9 | 10.5 | 1.7 | 3.7 |
| Do not know drug effects | males | 96.2 | 90.6 | 88.6 | 97.1 | 93.7 |
| | females | 100 | 97.9 | 90.2 | 98.8 | 97.6 |
| | in total | 97.9 | 96.1 | 89.5 | 98.3 | 96.3 |

It should also be noted that students from urban areas are more prone to alcohol consumption. Thus, it was determined that 2.6 % of students from urban areas and 0.6 % of students from rural areas drank alcohol regularly; 5.2 and 2.9 % – two or three times a week respectively; 20.6 and 17.0 % – once a week respectively (Table VI).

The main reasons defined that encourage students to drink alcohol were resting in a company (40.6%), meeting with friends (33.2 %), the desire to relax (9.0 %), boredom (4.2 %), meeting with parents (1.4 %). Only 39.4 % of students did not feel the need to drink alcohol for their leisure and recreation (Table VII). The research showed that students were involved in alcohol consumption not in families but communicating with their peers while watching movies, attending various parties, discos, offering alcoholic beverages with exotic names and bright advertising.

According to scientists [23], blood does not retain alcohol, it is accumulated in the cells of the central nervous system, liver, and heart. The breakdown products of alcohol can stay in these organs from 13 to 15 days. The repeated use of alcohol makes it even longer. The frequent alcohol consumption involves it in muscle metabolism and alcohol becomes its constant participant, especially in brain tissue. The first signs of chronic alcoholism are a strong craving for alcohol, increased endurance to alcohol, and a hangover. Severe alcohol intoxication kills about 20,000 nerve cells. Alcohol abuse is one of the leading causes of death directly and indirectly, especially for men of working age. According to the WHO, the

mortality rate among alcohol abusers is 2-4 times higher than among the general population [24].

Unfortunately, some young students also use drugs. The male students of the first year of study were found to use drugs, in contrast to the students of the 2nd – 4th years. This fact gives grounds to claim that they had already used drugs before entering HEI. The second-year female students were also involved in drug consumption, the number of which accounted for 2.4 % in the 4th year of study (Table VIII). The obtained data showed that 4.7 % of students used or tried drugs, 3.7 % certainly knew their effects, which also indicated that students used or tried drugs and experienced their effects.

In addition to the above-mentioned negative factors affecting the health of young people, there is also the use of psychotropic substances, which are not classified as narcotic but form a drug addiction and make a young person drug-addicted quickly. Drug addiction and substance abuse, as well as alcoholism, are characterized by three main features: mental and physical dependence on narcotic and toxic substances, as well as addiction to them. Alcoholism develops these symptoms gradually, whereas toxic substances and drug addiction – extremely quickly. Physical dependence is the patient's condition, which is characterized by intense physical and mental disorders if the substances consumption is stopped. An injection of a toxic drug can alleviate these disorders.

An intake of toxic substances through the respiratory system is one of the most dangerous ways because in addition to the general toxic effects on the whole body, they have a destructive influence on the cells of the bronchial epithelium. This leads to inflammation of the respiratory system. Besides, toxic substances have a dangerous impact on the cardiovascular system, metabolic processes, liver, central nervous system, etc. The consumption of drugs and toxic substances causes a degradation of personality, reduces intellectual abilities, leads to physical and mental exhaustion, emotional imbalance, the loss of moral attitudes and values. It should also be noted that new temptations have become widespread for young people recently, including gambling, Internet addiction, loss of volitional behavior, which have a significant negative impact on the health and general life of students.

## DISCUSSION

Nowadays, there are large number of diseases, which are directly conditioned by the imbalance of the actual needs of the individual and anthropogenic needs. Anthropogenic needs are considered to be the needs arising from human nature, a man as a biosocial phenomenon. Anthropogenic needs do not include the need for tobacco, alcohol, drugs, toxic substances, etc. The contradictions between the needs that a person creates in the process of life and those that are necessary and sufficient for the life and development often lead to infectious diseases, heart attack, insult, cancer and a whole range of socio-psychological imbalances [25, 26].

The main anthropogenic need is the need for life. The system of human needs distinguishes three main groups of anthropogenic needs [27, 28, 29]:

1. Vital needs ("existence") are a state of the human body caused by the internal imbalance of metabolism and homeostasis with changing constants of the internal environment, and biological imbalance with the external world. At the biological level, this group includes the following needs: food, water, air, light, temperature and climate, sexual partner, self-preservation, etc. At the social level, these are the needs for clothing, family, and housing. It also includes sanitary, household, and environmental needs..

2. Emotional needs ("satisfaction") are a certain state of mind caused by unsatisfied emotional attitudes to objects and phenomena of the world. The basis of emotional needs are the emotional states, which are revealed as feelings (aesthetic and communicative needs, the need for love and friendship).

3. Rational needs ("improvement") are controlled and maintained by volitional powers. They are aimed at achieving a conscious personal or social goal (educational, cognitive, intellectual, aesthetic, legal, self-improving, etc.).

Thus, the paper reveals the impact of bad habits and health needs on the HEI students' health. It proved that smoking, alcohol, drugs, and various destructive health practices are widespread among young students. The study found that very few health technologies are currently being introduced in the student environment.

Obviously, such alarming affairs require supplementing the system of measures to eliminate students' bad habits through physical education. This can be facilitated by the involvement of young students in independent physical exercises, sports, tourism, interesting leisure, etc. In order to make a person change one's way of life, the measures that would touch one's consciousness should be taken. This encourages a change in behavior, getting rid of addictions. The most efficient means of influencing consciousness are interactive technologies, i.e. those that influence several human signaling systems. Interactive technologies include modern video technologies with the use of computer programs – websites, video films, activating the need for a healthy lifestyle, the desire for beauty, harmony, preservation of life [30]. In addition, it is necessary to strengthen educational work and advocacy of the importance of a healthy lifestyle among young students, to create clubs for interesting leisure, to expand sports and cultural events, and introduce incentives for a healthy lifestyle. Therefore, in order to meet all needs, there must be a high standard of living, and it is not possible without a sufficient level of health. Poor health is characterized by reduced defenses and the body's resistance to the negative effects of the environment, which leads to disease. Diseases cause disorders of homeostasis, i. e. changes in much of the parameters of the internal environment, which leads to discomfort, and ultimately to pain and suffering. Thus, there is a chain reaction of interrelated changes, the initial link of which is homeostasis disorders, and the final – painful sensations. The changes in homeostasis are the primary link, a trigger for various functional issues caused by the

**Table IX.** The indicators of students' compliance with sleeping schedule (n=647), %

| Sleep duration | Gender | The year of study | | | | Total % |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | |
| Less than 7 hours | males | 22.9 | 34.4 | 17.1 | 14.7 | 23.9 |
| | females | 20.7 | 19.9 | 13.7 | 25.9 | 20.5 |
| | in total | 21.9 | 23.5 | 15.1 | 22.7 | 21.8 |
| 7–8 hours | males | 61.0 | 54.7 | 65.8 | 61.8 | 60.1 |
| | females | 51.2 | 69.6 | 76.5 | 58.8 | 64.6 |
| | in total | 56.7 | 65.9 | 72.1 | 59.7 | 62.9 |
| More than 8 hours | males | 16.1 | 10.9 | 17.1 | 23.5 | 16.0 |
| | females | 28.1 | 10.5 | 9.8 | 15.3 | 14.9 |
| | in total | 21.4 | 10.6 | 12.8 | 17.6 | 15.3 |

**Table X.** The students' compliance with sleep schedule (n=647), %

| Go to bed | Gender | The year of study | | | | Total % |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | |
| Before 10 p.m. | males | 3.8 | 3.1 | – | 8.9 | 3.8 |
| | females | 9.8 | 6.8 | 3.9 | 9.4 | 7.6 |
| | in total | 6.4 | 5.9 | 2.3 | 9.2 | 6.2 |
| After 10:30 p.m. | males | 16.2 | 15.6 | 31.4 | 17.6 | 18.5 |
| | females | 31.7 | 29.3 | 33.3 | 28.2 | 30.0 |
| | in total | 23.0 | 25.9 | 32.6 | 25.2 | 25.8 |
| After 11 p.m. | males | 52.4 | 42.2 | 42.9 | 55.9 | 48.7 |
| | females | 34.1 | 47.1 | 51.0 | 38.8 | 43.4 |
| | in total | 44.4 | 45.8 | 47.7 | 43.8 | 45.3 |
| After the midnight | males | 27.6 | 39.1 | 25.7 | 17.6 | 29.0 |
| | females | 24.4 | 16.8 | 11.8 | 23.5 | 19.0 |
| | in total | 26.2 | 22.4 | 17.4 | 21.8 | 22.7 |

disease. The temperature rise, increased heart rate, rapid breathing, muscle weakness and apathy, changes in blood pressure and blood composition are secondary changes aimed at neutralizing disorders of homeostasis. According to the mechanism of their origin and the orientation, these changes have a protective character. All these changes are accompanied by a deterioration of the emotional state. The research of homeostasis and reactions aimed at compensating for its disorders, it is established that homeostasis, functional capabilities of the organism, well-being and quality of life are closely related [25, 31].

The scientists [32, 33] note that the body is arranged in such a way that the changes in any organ cause changes in the parameters of other organs. These secondary changes are aimed at compensating for changes in homeostasis and stabilize the internal environment of the organism. Thus, the organism seeks to restore the impaired quality of life. The most common example of such recovery is a good sleep after a hard day. During sleep there the spent energy potential of an organism is restored, numerous changes in a metabolism caused by physical and mental activity occur. As a result, homeostasis is stabilized, which is revealed in improved well-being, the restoration of working capacity and health.

Therefore, compliance with sleeping schedule is one of the most important needs, which can significantly influence the efficiency and well-being of a student throughout the day. At the same time, research showed that 21.8 % of students slept less than 7 hours that did not meet daily needs. A significant part (15.3 %) of students spent more than 8 hours on sleep (Table IX).

The analysis of students' sleep schedule showed that most students did not follow a daily routine at all. A rational daily routine creates optimal conditions for the activity and recovery of the body and helps to increase physical and mental performance. It is conditioned by the fact that the correct and clear daily routine creates a certain rhythm of the organism activity; as a result, a student can perform various types of work most effectively at a certain time. Strict compliance with the daily routine is also a good means of developing organization, independence, will and it teaches a conscious discipline.

The scientists [8, 20] note that owing to different living, working and studying conditions, household and individual characteristics, there can be no single daily routine for everyone. But the basic provisions must be followed in any case. First, the student's daily routine should provide the performance of various kinds of activity in precisely defined time; the correct alternation of educational activities, physical exercises and rest; regular meals at the same time; the optimal time for preparing homework and physical training; long and good quality sleep. If a student does not go to bed on time, one can not get up early and start his/her day efficiently. Table X shows that 6.2 % of

students went to bed before 10 p.m., 25.8 % – after 10:30 p.m., 45.3 % – after 11 p.m., 22.7 % – after the midnight. The number of the students' sleep hours is also affected by the distance of their place of residence from the HEI, the amount of time spent on the way, breakfast, etc.

That is why the introductory physical education classes, especially for first-year students, should pay attention to the organization of the daily routine, help students navigate new circumstances, plan their time according to actual living conditions in the following way: morning toilet (gymnastics, shower (wash), personal body care); breakfast (without haste); studying, and recovery activities if possible in the morning; lunch followed by a break; mental work (in the library, at home, laboratories, etc.); exercises or sports (followed by active recovery procedures); dinner (easily digestible food); free time; sleep (sufficient) [34].

It is necessary to accustom organism to a certain system: to get up and go to bed at the same time; to work out from 5 to 7 p.m. This will lead to a physiologically correct mode of higher nervous activity and promote the development of certain behavior reflexes. As a result, it makes it possible to achieve a balance between the organism and the environment [8].

At the same time, many scientists [29, 31] claim that living in unfavorable hygienic conditions, without following a rational daily routine, personal hygiene rules leads to poor health and a sharp deterioration of health. The student's personal hygiene includes a wide range of issues related to a rational daily routine, body and dental care, compliance with the hygienic conditions of study and life, etc. The knowledge of the rules of personal hygiene is necessary for every student. This is especially important for student-athletes because strict compliance with these rules helps to improve health, increase the efficiency of training. Body care includes hygienic procedures for skin, hands, feet, mouth care, etc.

Almost every student experienced situations of homeostasis disorders associated with pain and activity restrictions. Even a light angina or cold cause discomfort or pain. Of course, a sore throat caused by inflammation and swelling in the tonsils is unpleasant. The students attribute the reason for the decline in quality of life to it. However, neither this pain nor the inflammatory process in the lymph glands, nor the temperature rise is the cause of the disease. These phenomena are the body's response to an adverse situation that has arisen because of disease-causing microbes. In most cases, such phenomena occur as a result of increased activity of microbial flora because of a decrease in protective forces that can be caused by stress, bad habits, etc. [32]

The next health-improving issue for the students' productive life that experts [10, 13, 35] advice is the elimination of various excesses in the organism. These are accumulated substances that become a burden or pathogen for the organism, the conditions or processes that go beyond the norm and worsen the body's vitality. The most typical example is overweight or obesity. There are two types of fat: intracellular, which is a mandatory component of the protoplasm of human cells, and extracellular. A small amount of extracellular fat should be placed in a thin layer (up to 2-3 mm) under the skin. The value of body fat is beyond doubt. However, an increased amount of extracellular fat in the organism creates a number of factors that negatively affect the viability of students, significantly reducing their health. An excessive amount of fat in the organism makes a person not energetic and tired, causes headaches, depression. The studies show that there is a link between obesity and health problems. Overweight people are more likely to have circulatory diseases, malignant tumors, diabetes. There is a relationship between the amount of excess fat in the organism and reduced life expectancy [25, 31].

An important reserve for updating the educational process is applying health technologies in the process of physical education, the introduction of which will provide students with knowledge, skills, and abilities to manage individual health, to deal with bad habits, and prepare them for highly qualified professional activities.

## CONCLUSIONS

1. The compliance with the regime of study and rest, nutrition, sleep, regular exercise helps to get rid of bad habits, which are usually accompanied by an inert state, reduced efficiency and reaction speed. At the same time, the system of education at HEI does not form a proper motivation for a healthy lifestyle. Most students know that smoking, alcohol, and drugs are bad but they can't or don't want to get rid of those bad habits. The viability and health of students largely depend on the student's behavior, which forms emotions that influence metabolism and energy, which provides a flow of non-specific (from the physical environment) and specific (from social conditions) information. All these influences significantly change the objective basis of health – viability. Forming various functional states of the organism, the students' behavior dramatically changes the potential of health.
2. The problem of health formation is becoming an important component of almost all modern pedagogical innovative technologies. To ensure efficient cooperation between teachers and students, health should be considered as a means of daily life, as a positive part of life, emphasizing social and personal resources, as well as the physical capabilities of each student. It was found that the environment of students is unfavorable for the activation of the healthy lifestyle components; it contributes to the spread of bad habits, the consequences of which students do not realize. Many students do not have a need to take care of their own health. Such students rely on their youth, the activities of medical institutions, and neglect such efficient and cost-effective means of rehabilitation as physical culture and sports.

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ORIGINAL ARTICLE

# EVALUATION OF BONE RESORPTIVE POTENTIAL IN THE TREATMENT OF GENERALIZED PERIODONTITIS

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## ABSTRACT

**The aim:** Is to study dynamics of resorptive potential of bone tissue by indicators of protease and antiprotease systems in the process of treatment generalized periodontitis in patients with age-related osteoporosis and without osteoporotic changes in the skeleton.

**Materials and methods**: In 102 patients, before the start of treatment of generalized periodontitis and in 2, 4 and 12 weeks, the concentration of pro-resorbing matrix metalloproteinases: stromelysin (MMP-3), collagenase (MMP-8) and gelatinase (MMP-9) were determined in blood plasma and mixed oral fluid. The antiresorbtive potential of bone tissue was evaluated by the concentration of a tissue inhibitor of metalloproteinases (TIMP-1) in plasma. The general antiprotease activity was determined by the activity of α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG).

**Results:** The most significant changes are recorded for the content of MMP-9 in blood and oral fluid, regardless of the presence of systemic disorders of bone metabolism ($P < 0.05$). Concentration of MMP-8 is significantly increased in blood plasma and oral fluid in accordance with the severity of the disease and in the course of treatment ($P < 0.05$). The observed increase in the ratio of MMP-8 to TIMP-1 and MMP-9 to TIMP-1 in patients with generalized periodontitis, complicated by systemic osteoporosis ($P < 0.05$), indicates an imbalance of the protease-antiprotease system.

**Conclusions:** The resorptive potential of bone tissue in patients with generalized periodontitis allows us to correctly choose treatment tactics and to prevent the development of complications.

**KEY WORDS:** periodontal diseases, osteopenia, matrix metalloproteinases

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## INTRODUCTION

Generalized periodontitis (GP), associated with a significant prevalence among the population, is a multifactorial disease that develops independently or as a result of systemic diseases [1]. The rate of progression of GP is dependent on bone systemic condition, particularly, degree of mineralization and metabolism [2]. The bone resorptive potential is determined mainly by the activity of osteoclasts and osteoblasts, but significant contribution to the destruction of bone matrix is made by periodontal fibroblasts, monocytes, macrophages and others. In the focus of inflammation, immune cells accumulate and secrete cytokines and proteases, thereby causing the release and activation of matrix metalloproteinases (MMPs), that are the key to the resorption of bone matrix [3].

Activity MMPs in physiological conditions is regulated with specific tissue inhibitors (TIMPs). Their concentration characterizes the antiprotease activity of biological fluids. Thus, TIMP-1 and TIMP-2 can inhibit the activity of all known MMPs. TIMP-3 and TIMP-4 are responsible for cell differentiation in extracellular matrix. TIMP-1 is a soluble inducible inhibitor with a wide spectrum of action, preferably produced by cells of the connective tissue matrix; and it is contained in bone tissue in a large quantity. However, the activated proteinases can be free to go out into the bloodstream and other biological fluids, affecting on the homeostasis of the organism. At this level of the regulation of protease-antiprotease balance, hemostatic antiprotease system is connected. Among plasma protease inhibitors, α2- macroglobulin is the most important; it reduces the activity of soluble MMPs [4].

However, it is still not clear what metalloproteinase is the most of all associated with bone resorption in inflammatory destructive processes in periodontal tissues, what value of TIMP-1 and protease inhibitors can be indicators of antiprotease system, in the development of inflammatory and destructive process in periodontal tissues. In our opinion, this kind of research has to have practical output in creating therapeutic and diagnostic methods in the complex treatment of disease; and it prompted us to carry out presented work.

## THE AIM

The aim of the study is examination of the dynamics of bone resorptive capacity according the indices of protease and antiprotease systems during the treatment of GP of the II-III degree of severity in patients with age-related osteoporosis and without osteoporotic changes in bone system.

**Table I.** Indicators of protease and antiprotease systems in biological fluids in research groups before treatment (M ± m)

| | Indicator | | Control | Comparison group (CG) | | Basic group (BG) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | I | II | I | II | III |
| protease | MMP-3 µg / l | blood plasma | 24.65 ± 4.78 | 29.24 ± 7.43 | 33.38 ± 9.64 *** | 43.56 ± 7.49 *** | 42.80 ± 6.61 *** | 47.62 ± 7.55 *** |
| | | saliva | 3.65 ± 0.57 | 3.85 ± 0.74 | 3.93 ± 0.86 | 4.44 ± 1.18 | 8.40 ± 1.38 *** | 8.45 ± 1.88 *** |
| | MMP-8 µg / l | blood plasma | 3.29 ± 0.59 | 3.68 ± 0.99 | 4.24 ± 0.82 *** | 2.58 ± 0.76 ** | 4.31 ± 0.57 *** | 5.20 ± 0,99 *** |
| | | saliva | 0.63 ± 0.14 | 1.17 ± 0.25 *** | 1.28 ± 0.21 *** | 0.75 ± 0.27 | 1.01 ± 0.18 *** | 1.29 ± 0.26 *** |
| | MMP-9 µg / l | blood plasma | 38.05 ± 6.13 | 50.9 ± 5.79 *** | 52.79 ± 5.83 *** | 41.49 ± 6.92 | 57.86 ± 7.05 *** | 60.97 ± 8.09 *** |
| | | saliva | 13.34 ± 2.26 | 21.74 ± 4.35 *** | 24.46 ± 5.24 *** | 15.58 ± 4.93 | 26.52 ± 4.94 *** | 28.80 ± 5,90 *** |
| | TTA, µM / min / ml | plasma blood | 0.51 ± 0.05 | 0.54 ± 0.07 | 0.65 ± 0.05 *** | 0.50 ± 0.04 | 0.69 ± 0.08 *** | 0.79 ± 0.12 *** |
| antiproteases | TIMP-1 µg / l | plasma blood | 105.60 ± 7.85 | 58.22 ± 6.51 *** | 131.58 ± 11.53*** | 112.49 ± 13.71 | 89.70 ± 14.55 *** | 81.95 ± 9.34 *** |
| | | saliva | 0.79 ± 0.14 | 1.54 ± 0.30 *** | 1.60 ± 0.29 *** | 1.05 ± 0.21 *** | 1.23 ± 0.18 *** | 0.70 ± 0,18 |
| | α1-AT, µM / min / ml | blood plasma | 35.89 ± 4.11 | 58.22 ± 6.51 *** | 46.98 ± 3.58 *** | 46.73 ± 4.02 *** | 50.23 ± 4.67 *** | 49.64 ± 6.19 *** |
| | | saliva | 1.73 ± 0.32 | 2.35 ± 0.45 *** | 2.57 ± 0.29 *** | 1.71 ± 0.50 | 1.02 ± 0.15 *** | 1.21 ± 0.34 *** |
| | α2-MG, µM / min / ml | blood plasma | 6.71 ± 0.70 | 9.16 ± 0.88 *** | 9.98 ± 1.07 *** | 7.03 ± 1.30 | 6.82 ± 1.31 | 5.09 ± 0.52 *** |
| | | saliva | 0.77 ± 0.09 | 0.88 ± 0.12 *** | 0.52 ± 0.08 *** | 0.69 ± 0.13 * | 0.69 ± 0.14 * | 0.55 ± 0.06 *** |

Notes. 1. n = 30 for all research groups. 2. * - P < 0.05, ** - P < 0.01, *** - p < 0.001 compared with control group.

## MATERIALS AND METHODS

The comprehensive study and treatment of 120 patients with GP, equally men and women, with average age 59.2 ± 5.4 years, was conducted. The diagnosis was determined by clinical and radiological data [1].

The state of bone tissue was determined by the results of the study of bone mineral density (BMD) by the method of two-energy x-ray absorptiometry using the apparatus Lunar Prodigy. The diagnosis of osteoporotic changes in the skeleton was held by the WHO recommendation by the T-criterion.

Among selected patients with GP, 60 persons had normal bone mineral density, and the remaining 60 ones had age osteoporotic changes in bone tissue. Additionally, the indicators were studied for 30 persons with age-related osteoporosis, whom are not diagnosed GP, and for 30 persons with intact periodontal tissues and without osteoporotic changes.

Criteria for exclusion from the study were receiving drugs with mineral components, the presence of injuries and inflammatory diseases of skeleton and joints. Group of research (basic and comparison) were formed identical in age and gender.

The I basic group (BG) consisted of 30 persons with concomitant age osteoporosis, whom are not diagnosed with inflammatory and destructive changes in periodontal tissues. The II BG included 30 patients with GP of the II degree of severity, chronicity. The III BG included 30 patients with GP of the III degree of severity, chronicity. The criteria for inclusion in the BG was presence of concomitant age osteoporosis provided there is no history of somatic pathology affecting the mineral density of the skeleton.

In turn, the I comparison group (CG) consisted of 30 patients with GP of the II degree of severity, chronicity. The II CG consisted of 30 patients with III degree of severity of disease. All patients, included CG, did not have osteoporotic changes in skeleton bone. The control group included 30 relatively healthy persons without both inflammatory and destructive changes in periodontal tissues and osteoporotic changes in the skeleton.

Patients with GP received combined treatment according to standard protocol [1]. The material for biochemical studies was plasma (serum) of blood and saliva before treatment of GP, and in 2, 4 and 12 weeks after its beginning.

For the comprehensive assessment of the activity of proteolytic processes in the bone tissue, it was studied the concentration of MMPs of three main subclasses, participating in the resorption of bone matrix, namely stromelizyn (MMP-3), collagenase (MMP-8) and gelatinase (MMP-9) in blood plasma using R&D Diagnostics Inc. kit (USA). Also, trypsin-like total activity (TTA) of plasma, which is based on the these enzymes' decomposition colorless synthetic substrate N-benzoyl-DL-arginine-4-paranitroanilinohydrochloride with the formation of p-nitroaniline with yellow colour, were determined. The degree of color were recorded at a wavelength of 410 nm using a photometer 2000 Human (Human, Germany). Quantitative evaluation TTA in plasma was performed using the calibration graph, where the various concentrations of aniline, the final cleavage product of trypsin-like enzymes, were used as standard solutions. Specific trypsin-like activity in blood plasma was determined in µmol / minute of fermented substrate in terms of 1 liter of serum (µM / minute / l).

The anti-resorptive potential of bone tissue was evaluated by the concentration of MMPs tissue inhibitor TIMP-1 in blood plasma using the R&D Diagnostics Inc. kit (USA).

Based on the fact that the concentration of MMPs does not fully reflect the activity of degradation of the intercellular matrix, which is mainly due to the resistance to proteinase specific inhibitors, we additionally determined the total anti-proteolytic activity of biological fluids according

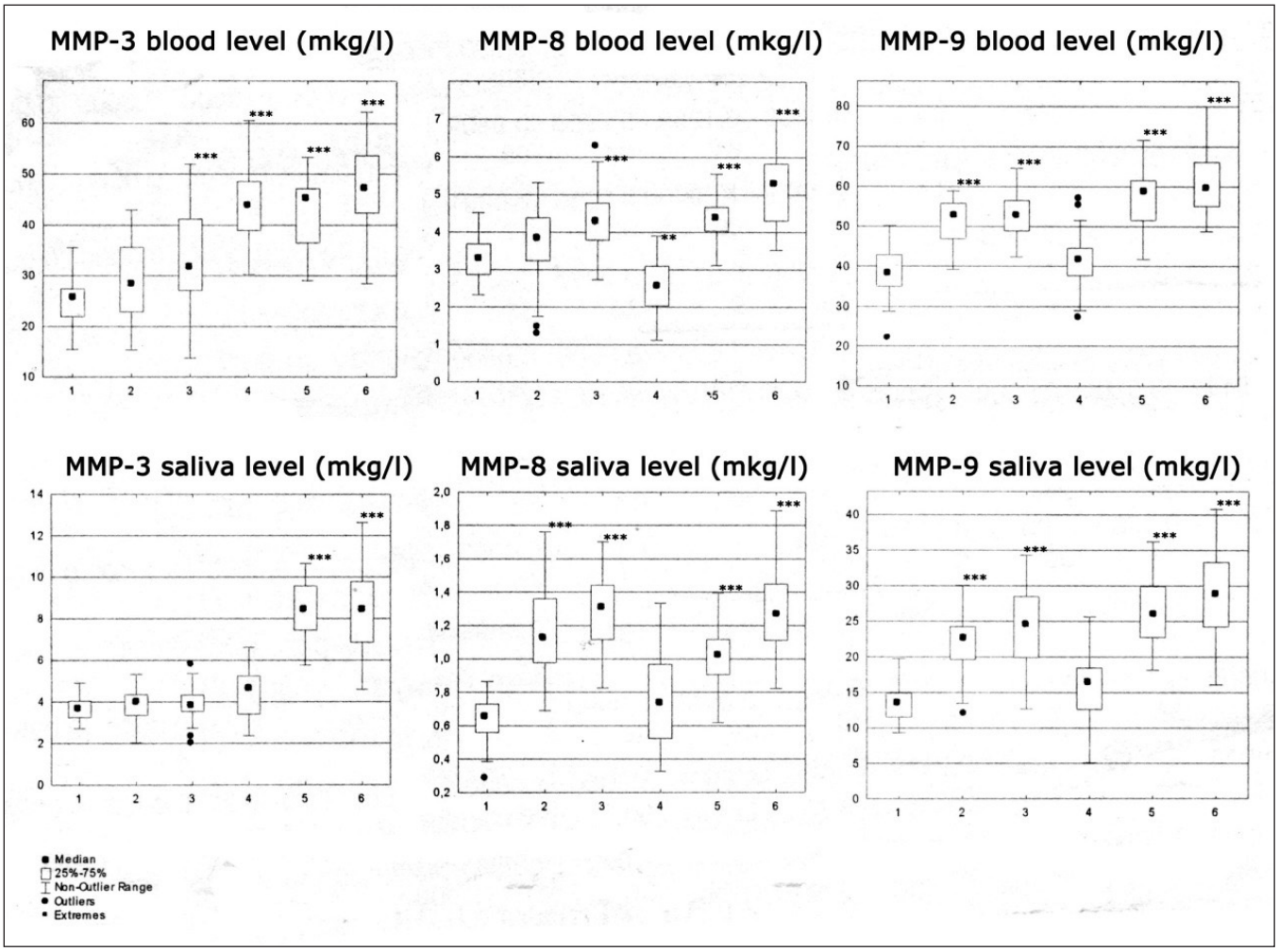


**Fig.1.** Concentration of MMPs in biological fluids (1 - control group; 2 – the I CG; 3 – the II CG; 4 – the I BG, 5 – the II BG, 6 – the III BG) before treatment (M ± m)

the activity of α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG) by the Nartikova & Pashina's method [5]. It should also be noted that prior to the studies, samples were normalized by the total protein concentration determined by the method [6].

Statistical analysis was performed using the package Statistica 8.0 (Statsoft Inc., USA).

## RESULTS

Systematic content of MMPs in blood plasma and saliva varied depending, firstly, on the stage of GP and, secondly, on the presence of concomitant osteoporosis, increasing according to the severity of the pathological process in periodontal tissues and its complications under systemic disorders of bone metabolism (Table I).

In patients of the BG, nonsignificant increase in the level of MMP-3 in plasma was observed, while the local concentration of the MMPs in saliva varied significantly ($P < 0.001$). At the same time, in patients of BG, significant differences of MMP-3 concentration in plasma and saliva, compared to each other, were not observed ($P > 0.05$).

In patients with GP, the MMP-8 level significantly increased in plasma ($P < 0.05$), most notably against background of systemic osteoporosis and the third degree of the destructive and inflammatory process in periodontal tissues ($P < 0.001$). Regarding the MMP-8 content in saliva, this index showed the same, but more pronounced, dynamics, that in plasma. Its differences with values of the control group proved to be reliable in patients with GP in all observation groups (Figure 1).

According to the results, the MMP-9 concentration also increased in the blood and saliva of patients with GP, but the differences of the indices for the saliva in GP of the II degree of severity were less pronounced ($P < 0.001$).

Thus, the analysis of the study of MMPs proved representativeness of content MMP-8 and MMP-9 in both blood plasma and saliva, regarding the description of the activity of the destructive process in the periodontium. However, as expected, the most significant changes were found in MMP-8 concentrations in biological fluids studied in the patients of the basic and comparison groups in GP of the III degree of severity ($P < 0.001$). Changes in the index of saliva, unlike MMP-9, were registered in all variants of GP. The level of MMP-3 is more related to osteoporotic bone lesions and was not significant in assessing the severity of GP.

**Table II.** Indicators of protease and antiprotease systems in plasma in research groups during treatment (M ± m)

| Group | | | Indicator | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | MMP-3 µg / l | MMP-8 µg / l | MMP-9 µg / l | TTA, µM/min/ml | TIMP-1 µg / l | α1-AT, µM/min/ml | α2-MG, µM / min / ml |
| | Control | | 24.65 ± 4.78 | 3.29 ± 0.59 | 38.05 ± 6.13 | 0.51 ± 0.05 | 105.60 ± 7.85 | 35.89 ± 4.11 | 6.71 ± 0.70 |
| Comparison (CG) | I | before treatment | 29.24 ± 7.43 | 3.68 ±0.99 | 50.90 ± 5.79 | 0.54 ± 0.07 | 135.91 ± 10.08 | 58.22 ± 6.51 | 9.16 ± 0.88 |
| | | 2 weeks | 27.82 ± 7.07 | 3.55 ± .96 | 46.83 ± 5.33 | 0.53 ± 0.07 | 139.57 ± 10.35 | 50.51 ± 5.65 *** | 7.22 ± 0.69 |
| | | 4 weeks | 27.23 ± 6.92 | 3.22 ±0.87 | 45.51 ± 5.18 | 0.52 ± 0.07 | 127.05 ± 9.42 | 37.64 ± 4.21 *** | 8.06 ± 0.77 *** |
| | | 12 weeks | 28.02 ± 7.12 | 3.79 ±1.02 | 46.10 ± 5.24 | 0.55 ± 0.07 | 118.25 ± 8.77 *** | 38.89 ± 4.35 *** | 7.34 ± 0.70 ** |
| | II | before treatment | 33.38 ± 9.64 | 4.24 ±0.82 | 52.79 ± 5.83 | 0.65 ± 0.05 | 131.58 ± 11.53 | 46.98 ± 3.58 | 9.98 ± 1.07 *** |
| | | 2 weeks | 28.43 ± 8.21 | 3.90 ±0.76 | 50.06 ± 5.53 | 0.58 ± 0.05 * | 130.59 ± 11.44 | 37.17 ± 2.83 *** | 9.76 ± 1.04 |
| | | 4 weeks | 26.29 ± 7.59 * | 3.42 ± 0.66 ** | 49.73 ± 5.50 | 0.59 ± 0.05 | 128.26 ± 11.24 | 38.68 ± 2.94 *** | 8.89 ± 0.95 ** |
| | | 12 weeks | 27.43 ± 7.92 | 3.63 ± 0.70 | 43.57 ± 4.82 *** | 0.55 ± 0.05 *** | 115.88 ± 10.15 *** | 38.59 ± 2.94 *** | 8.02 ± 0.86 *** |
| Basic (BG) | I | before treatment | 43.56 ± 7.49 | 2.58 ± 0.76 | 41.49 ± 6.92 | 0.50 ± 0.04 | 112.49 ± 13.71 | 46.73 ± 4.02 | 7.03 ± 1.30 |
| | II | before treatment | 42.80 ± 6.61 | 4.31 ± 0.57 | 57.86 ± 7.05 | 0.69 ± 0.08 | 89.7 ± 14.55 | 50.23 ± 4.67 | 6.82 ± 1.31 |
| | | 2 weeks | 40.15 ± 6.20 | 4.03 ± 0.53 | 52.28 ± 6.37 * | 0.61 ± 0.07 ** | 92.26 ± 14.97 | 45.64 ± 4.24 * | 6.97 ± 1.34 |
| | | 4 weeks | 41.32 ± 6.38 | 3.75 ± 0.50 | 49.39 ± 6.02 *** | 0.63 ± 0.07 | 107.34 ± 17.41 *** | 45.71 ± 4.25 * | 7.12 ± 1.37 |
| | | 12 weeks | 38.77 ± 5.99 | 3.49 ± 0.46 ** | 47.46 ± 5.78 *** | 0.60 ± 0.07 *** | 103.43 ± 16.78 ** | 39.1 ± 3.64 *** | 7.40 ± 1.42 |
| | III | before treatment | 47.62 ± 7.55 | 5.20 ± 0.99 | 60.97 ± 8.09 | 0.79 ± 0.12 | 81.95 ± 9.34 | 49.64 ± 6.19 | 5.09 ± 0.52 |
| | | 2 weeks | 45.73 ± 7.25 | 5.11 ± 0.97 | 55.98 ± 7.43 | 0.77 ± 0.11 | 96.5 ± 11.00 *** | 48.70 ± 6.07 | 5.29 ± 0.54 |
| | | 4 weeks | 43.60 ± 6.91 | 4.34 ± 0.82 ** | 47.68 ± 6.33 *** | 0.73 ± 0.11 | 108.21 ± 12.34 *** | 49.68 ± 6.19 | 5.47 ± 0.56 |
| | | 12 weeks | 41.47 ± 6.57 | 3.62 ± 0.69 *** | 45.72 ± 6.07 *** | 0.71 ± 0.11 ** | 114.53 ± 13.06 *** | 48.59 ± 6.06 | 5.89 ± 0.60 |

Notes. 1. n = 30 for all research groups. 2. * - P < 0.05, ** - P < 0.01, *** - p < 0.001 compared with control group.

Data on changes in the concentration of MMPs in plasma and saliva during treatment are given in the Tables II and III. The level of MMP-3 remained unchanged in both biological fluids. The results obtained after treatment exceeded the control values almost twice (P < 0.01). The concentration of MMP-9, as a result of the treatment, decreased rather rapidly, especially in the blood plasma, more dynamically in the groups with GP of the III degree of severity (P < 0.05 compared to the concentration before treatment), but did not reach plasma parameters in the control group at the end of treatment. On the contrary, MMP-8 concentration was normalized in both saliva and blood plasma over the treatment period, and the final results were almost consistent with the control values (P < 0.05).

The values of the protease system (TTA) of the blood plasma also changed according to the degree of destruction of periodontal bone under the influence of local inflammatory-destructive process and systemic osteoporotic changes. Its highest values were obtained for patients with GP of the II-III degrees of severity on the background of age-old systemic osteoporosis (P < 0.001 when compared with control values), the lowest – for the II degree without systemic changes in the skeleton (P > 0.05 when compared with control values). However, this index was not indicative in the dynamics of treatment, the final results significantly exceeded the control values (P < 0.01).

The levels of α1-AT and α2-MG in the blood plasma, as indicators of the state of the antiproteinase system, are slightly increased in the CG (P < 0.05), and in the main group these indicators tend to decrease and do not differ significantly from the control group (P > 0.05). However, with GP of the III degree of severity in both groups, the activity of α2-MG in saliva decreased, which indicates a decrease in the local antiproteinase protection of periodontal tissues. Changes in these parameters during treatment occured, but without significant dynamics in patients with osteoporosis (P > 0.05), which limits their diagnostic value.

In addition, the release of MMPs in the presence of destructive phenomena in the bone system of general or only local origin, as discussed above, was offset by an increase in the concentration of TIMP-1, both in blood plasma and saliva (Table I), which was most noticeable in patients with GP of the II-III severity without osteoporotic changes. Regarding studied dynamics of TIMP-1 indices in the biological fluids, it was proved to be quite indicative of the inhibition of pathological processes in periodontium due to the treatment.

The ratio of MMPs concentration to the TIMP-1 in blood plasma and saliva was changing in different directions (Table IV). The most revealing was increased MMPs to TIMP-1 in saliva in patients with GP, complicated system osteoporosis (P < 0.001). This demonstrated a local imbalance of protease-antiprotease system. These indices grown most notably in GP of the III degree, in both the basic and the comparison group. The ratio MMP-3 to TIMP-1 in saliva increased only in BG, i.e.in patients with GP, occurring against the background of systemic osteoporosis. In blood plasma the ratio of MMP-3 to TIMP-1 was increased only in the BG and the ratio of MMP-8 and MMP-9 to TIMP-1 increased more than twice in groups with GP on the background of osteoporosis (P < 0.001).

**Table III.** Indicators protease and antiprotease systems in saliva in research groups during treatment (M ± m)

| Group | | | Indicator | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | MMP-3 µg / l | MMP-8 µg / l | MMP-9 µg / l | TIMP-1 µg / l | α1-AT, µM/min/ml | α2-MG, µM/min/ml |
| Control | | | 3.65 ± 0.57 | 0.63 ± 0.14 | 13.34 ± 2.26 | 0.79 ± 0.14 | 1.73 ± 0.32 | 0.77 ± 0.09 |
| Comparison (CG) | I | before treatment | 3.85 ± 0.74 | 1.17 ± 0.25 | 21.74 ± 4.35 | 1.54 ± 0.30 | 2.35 ± 0.45 | 0.88 ± 0.12 |
| | | 2 weeks | 3.80 ± 0.73 | 0.78 ± 0.17 *** | 20.33 ± 4.06 | 1.20 ± 0.23 *** | 2.16 ± 0.42 | 0.86 ± 0.12 |
| | | 4 weeks | 3.90 ± 0.75 | 0.71 ± 0.16 *** | 19.47 ± 3.89 | 1.13 ± 0.22 *** | 2.21 ± 0.43 | 0.81 ± 0.11 |
| | | 12 weeks | 3.66 ± 0.70 | 0.68 ± 0.15 *** | 17.12 ± 3.42 | 1.05 ± 0.21 *** | 2.04 ± 0.39 * | 0.79 ± 0.11 |
| | II | before treatment | 3.93 ± 0.86 | 1.28 ± 0.21 | 24.46 ± 5.24 | 1.60 ± 0.29 | 2.57 ± 0.29 | 0.52 ± 0.08 |
| | | 2 weeks | 5.36 ± 1.17 ** | 0.88 ± 0.14 *** | 20.47 ± 4.39 | 1.22 ± 0.22 *** | 2.50 ± 0.29 | 0.51 ± 0.08 |
| | | 4 weeks | 4.46 ± 0.97 | 0.79 ± 0.13 *** | 19.61 ± 4.20 * | 1.19 ± 0.21 *** | 2.08 ± 0.24 *** | 0.59 ± 0.09 |
| | | 12 weeks | 4.07 ± 0.89 | 0.68 ± 0.11 *** | 19.09 ± 4.09 *** | 1.03 ± 0.19 *** | 1.98 ± 0.23 *** | 0.64±0.10** |
| Basic (BG) | I | before treatment | 4.44 ± 1.18 | 0.75 ± 0.27 | 15.58 ± 4.93 | 1.05 ± 0.21 | 1.71 ± 0.50 | 0.69 ± 0.13 |
| | II | before treatment | 8.4 ± 1.38 | 1.01 ± 0.18 | 26.52 ± 4.94 | 1.23 ± 0.18 | 1.02 ± 0.15 | 0.69 ± 0.13 |
| | | 2 weeks | 7.93 ± 1.30 | 0.95 ± 0.17 | 22.51 ± 4.20 | 1.12 ± 0.16 | 0.98 ± 0.15 | 0.71 ± 0.13 |
| | | 4 weeks | 8.49 ± 1.40 | 0.92 ± 0.16 | 18.91 ± 3.52 *** | 1.03 ± 0.15 * | 1.17 ± 0.17 | 0.71 ± 0.13 |
| | | 12 weeks | 8.40 ± 1.38 | 0.84 ± 0.15 | 17.65 ± 3.29 *** | 1.02 ± 0.15 * | 1.30 ± 0.19 | 0.74 ± 0.14 |
| | III | before treatment | 8.45 ± 1.88 | 1.29 ± 0.26 | 28.80 ± 5.90 | 0.70 ± 0.18 | 1.21 ± 0.34 | 0.55 ± 0.06 |
| | | 2 weeks | 8.52 ± 1.90 | 1.23 ± 0.25 | 28.13 ± 5.77 | 0.69 ± 0.17 | 1.25 ± 0.35 | 0.56 ± 0.06 |
| | | 4 weeks | 8.56 ± 1.91 | 1.08 ± 0.22 ** | 27.62 ± 5.66 | 0.83 ± 0.21 | 1.33 ± 0.38 | 0.58 ± 0.06 |
| | | 12 weeks | 7.14 ± 1.59 ** | 0.97 ± 0.19 *** | 23.28 ± 4.77 *** | 0.86 ± 0.21 | 1.40 ± 0.40 | 0.62 ± 0.07 |

Notes. 1. n = 30 for all research groups. 2. * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared with control group.

According to the analysis of results for the basic and comparison groups for the purpose of evaluation of the total antiproteinase activity of blood plasma, in patients with GP of the II-III degrees of severity without osteoporotic changes, the antiproteinase activity was increased. On the contrary, under GP of the II-III degrees of severity with osteoporotic changes, the antiproteinase system was overloaded due to the significant activation of the proteinases, so the TTA in the BG tended to decrease.

## DISCUSSION

The study of MMP-3, MMP-8, MMP-9 and TIMP-1 content in patients with GP of the II-III degree of severity, including cases, complicated systemic osteoporosis, revealed their multiple changes depending on the severity of the disease and its treatment. In our opinion, that indicates the key role of disorders of the balance of protease and antiprotease systems of bone matrix in the development of periodontal pathology.

MMP-8 plays leading role in the destruction of periodontal tissues: it is a major destructive factor in the progression of GP, unburdened by disorders of bone metabolism.

In patients with GP in both research groups, the concentration of MMP-9 was significantly higher than normal. We consider that this is due to the high content of the cellular elements of the periodontal enzyme in the inactive form; the MMP-9 is activated by both cytokines and other proteinases. On the one hand, this indicates the greatest contribution to the destruction of periodontal tissues MMP-9, on the other hand – this MMP is a nonspecific indicator of inflammatory periodontal diseases and their complications.

At least, MMP-3 characterizes the destruction of periodontal tissues, in particular its bone component. Its activity is the greatest under the GP on the background of osteoporosis. While, if the process becomes more active, other proteinases are activated, which have a greater contribution to the destruction of the periodontal tissues. This is due to the lower content of MMP-3 in bone tissue and the activation of this MMP under the action of hormones and internal regulatory factors. However, it should be noted that the determination of MMP-3 is valuable in the diagnosis of systemic osteoporosis and the assessment of systemic activity of its course, as confirmed by the results of studies of precursors [7].

Despite the level of MMP-3, which is within the normal range of control and comparison individuals, the concentration of this MMP in the I BG is higher than normal, and in the II and III BG s it is significantly higher than normal ($P < 0.01$). This is indirectly indicative of the background course of osteoporotic changes in bone tissue and coincides with data from previous studies [8-9], which show the high value of MMP-3 in the processes of non-inflammatory destruction of the bone matrix. In this regard, the level of MMP-3 remains high enough in the II and III BG, despite the comprehensive treatment and elimination of the inflammatory component of osteodestruction. Also, these results indicate a latent course of osteodestruction and the achievement of temporary remission in the treatment of GP on the background of osteoporosis.

**Table IV.** Ratio of matrix metalloproteinases concentration to the TIMP-1 in biological fluids (M ± m)

| Biological fluids | Indicator | Control | Comparison group (CG) | | Basic group (BG) | | |
|---|---|---|---|---|---|---|---|
| | | | I | II | I | II | III |
| blood plasma | MMP-3 / TIMP-1 | 0.24 ± 0.05 | 0.22 ± 0.06 | 0.25 ± 0.07 | 0.39 ± 0.09 | 0.49 ± 0.11 | 0.59 ± 0.12 |
| | MMP-8 / TIMP-1 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.05 ± 0.01 | 0.06 ± 0.01 |
| | MMP-9 / TIMP-1 | 0.36 ± 0.07 | 0.38 ± 0.04 | 0.40 ± 0.06 | 0.37 ± 0.08 | 0.66 ± 0.10 | 0.75 ± 0.13 |
| saliva | MMP-3 / TIMP-1 | 4.83 ± 1.30 | 2.60 ± 0.75 | 2.55 ± 0.76 | 4.43 ± 1.52 | 6.96 ± 1.60 | 12.77 ± 4.15 * |
| | MMP-8 / TIMP-1 | 0.82 ± 0.21 | 0.78 ± 0.23 | 0.83 ± 0.22 | 0.74 ± 0.30 | 0.84 ± 0.19 | 1.92 ± 0.31 * |
| | MMP-9 / TIMP-1 | 17.57 ± 4.61 | 14.67 ± 4.39 | 15.71 ± 4.40 | 15.49 ± 6.76 | 22.05 ± 5.67 * | 43.2 ± 12.66 * |

Notes. 1. n = 30 for all research groups. 2. * – $P < 0.001$ compared with control group.

Describing the role of expression and activation of MMPs by signs that have been determined, it should be emphasized that normally the tissues do not contain active MMPs and the concentration of their precursors is at a minimal level. Thus, both stages of regulation are necessary for the accumulation in the bone tissue of the active form of the matrix.

TIMP-1 inhibits the activity of MMP-3, MMP-8, and MMP-9 in a 1:1 ratio, directly interacting with the active center of MMPs [10]. In the comparison group, the ratio of MMP-8 to TIMP-1 in the blood plasma increased in comparison with the control in 2.3 and 2.6 times, depending on the severity of the disease ($P < 0.01$). This was confirmed by the fact that with the activation of inflammation there is an increase in the concentration of MMP-8, "responsible" for the destruction of tissues of the periodontal complex, and the compensatory increase in the level of TIMP-1 was insufficient, which led to a violation of the balance of proteolytic activity. In addition, TIMP-1 can be inactivated by proteolytic enzymes – trypsin, chemotrypsin and neutrophil elastase – thus significantly increasing the activity of MMPs.

According to our observations, the ratios of MMPs to TIMP-1 in the blood plasma of the subjects were uninformative. In the BG, the ratio of MMPs to TIMP-1 in saliva increased significantly, indicating a pronounced local imbalance of the protease and antiprotease system, which causes the severity of periodontitis in patients with age-related osteoporosis and confirms the role of MMPs in the pathogenesis of disorders of the extracellular matrix.

Indicators of the antiproteinase system state were not indicative of the course as inflammatory-destructive phenomena in periodontal tissues. Although, we observed some changes that caused the activation of pathological phenomena. While the study of qualitative and quantitative characteristics of MMPs and their inhibitors represents a promising area of basic research that will allow the development of new approaches to the diagnosis and treatment of inflammatory-destructive periodontal diseases.

## CONCLUSIONS

In patients with GP, indicators of MMP-9 content in blood and saliva characterize the severity of inflammatory-destructive processes in bone tissue during generalization of the process. They are not indicative for evaluation of treatment effectiveness. The concentration of MMP-8 in patients with GP increases in plasma and saliva according to the severity of the disease; it changes also during the treatment. The increase in the ratio of MMPs to TIMP-1 in saliva in patients with GP, complicated by systemic osteoporosis, indicates a local imbalance of the protease-antiprotease system. The concentration of MMP-3 in saliva and blood plasma characterizes general changes in bone tissue, but is not representative of the activity of GP. In patients with GP, in saliva the level of α1-AT and α2-MG decreases, which indicates a decrease in local antiproteinase protection of periodontal tissue. In patients with GP, assessment of bone resorptive potential is to identify the leading pathogenetic mechanism of bone resorption at the current stage of disease and allows choosing the tactics of treatment: to prescribe the correct pharmacotherapy and to prevent the development of complications. Wherein, MMP-8 and TIMP-1 are the most significant markers of the inflammatory-destructive process in the periodontal tissues.

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ORIGINAL ARTICLE

# OCENA SYTUACJI FINANSOWEJ WYBRANYCH INSTYTUTÓW BADAWCZYCH NADZOROWANYCH PRZEZ MINISTRA ZDROWIA W LATACH 2014-2018

# ASSESSMENT OF THE FINANCIAL STANDING OF SELECTED RESEARCH INSTITUTES SUPERVISED BY THE MINISTER OF HEALTH BETWEEN 2014-2018

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## ABSTRACT

**The aim:** The purpose of this study is the analysis of the financial situation of the institutes supervised by the Minister of Health in the period 2014-2018.

**Material and methods:** The study group consisted of 10 institutes that conducted inpatient and / or outpatient medical activities in 2018. Data to analysis derived from financial statements of research institutes from 2014 to 2018. The selection of financial indicators to ratio analysis is based on Ministry of Health ordinance from 2017.

**Results:** The situation of the analyzed hospitals is difficult, with the difference between individual entities. The analyzed units were characterized by high net losses (9 out of 10 units generated a loss each year) low value of revenues in relation to costs and high share of remuneration in the structure of operating costs. There was no visible financial situation improvement per year. Simultaneously, in 2018 the difference between units were major: on a point scale 0 -70 ranged from: 51 points (73%) at the Institute of Physiology and Pathology of Hearing to 9 points (13%) at the Institute of Polish Mother's Health Center. The results are consistent with the conclusions of the Supreme Audit Office's reports, that baseline, medical institutes do not pursue a sustainable policy.

**Conclusions:** Based on the analyzed data, it can be concluded that the economic situation of these units in 2014-2018 is difficult and has not changed much compared to previous periods.

**KEY WORDS:** financial situation, indebtness, ratio analysis, research institute

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## WSTĘP

Problematyka oceny sytuacji finansowej publicznych świadczeniodawców usług zdrowotnych w polskim systemie ochrony zdrowia była analizowana w licznych publikacjach naukowych [1-8]. Potrzeba oceny i monitorowania sytuacji finansowej jest szczególnie istotna w kontekście zadłużenia publicznych świadczeniodawców. Ministerstwo Zdrowia systematycznie publikuje zagregowane dane o poziomie zadłużenia samodzielnych publicznych zakładów opieki zdrowotnej (SPZOZ). Według stanu na koniec 2019 r. poziom zadłużenia ogółem SPZOZ wyniósł 14,3 miliarda złotych, a poziom zobowiązań wymagalnych, których termin spłaty już minął, 1,9 mld złotych [9]. Dane te nie obejmują jednak zadłużenia podmiotów leczniczych funkcjonujących w formie instytutów badawczych, które stanowią szczególną formę organizacyjno-prawną. Funkcjonują one w oparciu o zapisy ustawy z dnia 30 kwietnia 2010 r. o instytutach badawczych [10]. Ich głównym celem jest prowadzenie badań naukowych i prac rozwojowych ukierunkowanych na wdrożenie oraz zastosowanie w praktyce. Minister Zdrowia nadzoruje 15 instytutów badawczych, z czego 10 prowadzi działalność z zakresu stacjonarnej i/lub ambulatoryjnej opieki zdrowotnej. Uczestniczą one w systemie ochrony zdrowia poprzez udzielanie świadczeń zdrowotnych, w powiązaniu z prowadzeniem badań naukowych i prac rozwojowych [11].

Najwyższa Izba Kontroli (NIK) kilkukrotnie podejmowała się analizy funkcjonowania instytutów badawczych, w tym również oceny ich sytuacji finansowej [14, 15, 17]. Raporty NIK opublikowane w roku 2012 i 2015 wskazały m.in. na trudną sytuację finansową instytutów badawczych nadzorowanych przez Ministra Zdrowia. Audytorzy podkreślali występowanie licznych zaniedbań w kontroli sytuacji finansowej ze strony organu tworzącego, brak właściwych mechanizmów zarządczych oraz pilną potrzebę

**Tabela 1.** Ogólna charakterystyka analizowanych podmiotów, według stanu na rok 2018.

| L. p. | Nazwa szpitala | Adres | Oznaczenie | Wartość majątku w 2018 (w mln) | Wartość przychodów ze sprzedaży w 2018 (w mln) | Liczba łóżek |
|---|---|---|---|---|---|---|
| 1 | Instytut Fizjologii i Patologii Słuchu | ul. Mochnackiego 10, Warszawa | IFiPS | 267,6 | 122,0 | 136 |
| 2 | Instytut Hematologii i Transfuzjologii | ul. Indihry Gandhi 14, Warszawa | IHiT | 180,2 | 258,8 | 193 |
| 3 | Instytut Gruźlicy i Chorób Płuc | ul. Płocka 26, Warszawa | IGiP | 138,7 | 125,3 | 256 |
| 4 | Instytut Matki i Dziecka | ul. Kasprzaka 17, Warszawa | IMiD | 141,9 | 142,9 | 201 |
| 5 | Instytut Geriatrii, Reumatologii i Rehabilitacji | ul. Spartańska 1, Warszawa | IGiR | 86,2 | 72,1 | 259 |
| 6 | Instytut Kardiologii | ul. Alpejska 42, Warszawa | IK | 187,5 | 235,0 | 325 |
| 7 | Instytut Centrum Zdrowia Matki Polki | ul. Rzgowska 281/289, Łódź | ICZMP | 296,0 | 265,8 | 813 |
| 8 | Instytut Psychiatrii i Neurologii | ul. Sobieskiego 9, Warszawa | IPiN | 182,0 | 146,1 | 555 |
| 9 | Instytut – Pomnik Centrum Zdrowia Dziecka | Al. Polskich Dzieci 20, Warszawa | IPCZD | 303,3 | 324,7 | 609 |
| 10 | Centrum Onkologii* | ul. Roentgena 5, Warszawa (główna siedziba instytutu) | CO | 1 146,0 | 1 269,1 | 1 205 |

*Wysoka wartość dla Centrum Onkologii i duża liczba łóżek wynika ze zsumowania wartości przychodów i majątku ośrodków w Warszawie, Krakowie i Gliwicach.
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

podjęcia działań restrukturyzacyjnych. W niniejszej pracy podjęto się analizy najnowszych dostępnych danych finansowych, obejmujących okres 2014-2018.

## CEL PRACY

Celem głównym przeprowadzonych analiz była ocena sytuacji finansowej wybranych instytutów badawczych nadzorowanych przez Ministra Zdrowia w okresie 2014-2018.

Cele szczegółowe objęły ocenę:

1. dynamiki i struktury majątku oraz źródeł finansowania;
2. dynamiki przychodów i kosztów;
3. dynamiki wartości wyniku finansowego i nadwyżki finansowej;
4. wskaźników finansowych w czterech obszarach: płynności finansowej, zadłużenia, efektywności i rentowności.

Powyższe analizy miały na celu odpowiedzieć na pytanie, czy nastąpiła istotna zmiana w sytuacji finansowej badanych jednostek w okresie ostatnich pięciu lat, dla których dostępne są dane.

## MATERIAŁ I METODY

Grupę badaną stanowiło 10 instytutów badawczych nadzorowanych przez Ministra Zdrowia, które prowadzą działalność stacjonarną i/lub ambulatoryjną (Tab. 1). Materiał źródłowy stanowiły sprawozdania finansowe za lata 2014-2018. Informację o liczbie łóżek w placówkach uzyskano z Rejestru Podmiotów Wykonujących Działalność Leczniczą i/lub ze stron internetowych placówek [12].

Zastosowane **metody badawcze** obejmują wstępną i pogłębioną analizę danych zawartych w sprawozdaniach finansowych. Analiza wstępna obejmuje analizę: dynamiki i struktury pozycji bilansu oraz rachunku zysków i strat. Analiza wskaźnikowa stanowi rozwinięcie analizy wstępnej. Zestaw wskaźników zastosowanych do oceny kondycji finansowej instytutów badawczych, pochodzi z Rozporządzenia Ministra Zdrowia z 12 kwietnia 2017 roku w sprawie wskaźników ekonomiczno-finansowych niezbędnych do sporządzenia analizy oraz prognozy sytuacji ekonomiczno-finansowej samodzielnych publicznych zakładów opieki zdrowotnej [13]. Dokument ten zawiera matrycę wskaźników przydatnych w ocenie i monitorowaniu sytuacji finansowej publicznych podmiotów leczniczych. Wskaźniki wymienione w rozporządzeniu podzielono na cztery kategorie: wskaźniki zyskowności, płynności, efektywności oraz zadłużenia, a wartościom poszczególnych indykatorów przypisano oceny punktowe (Tab. 2).

## WYNIKI

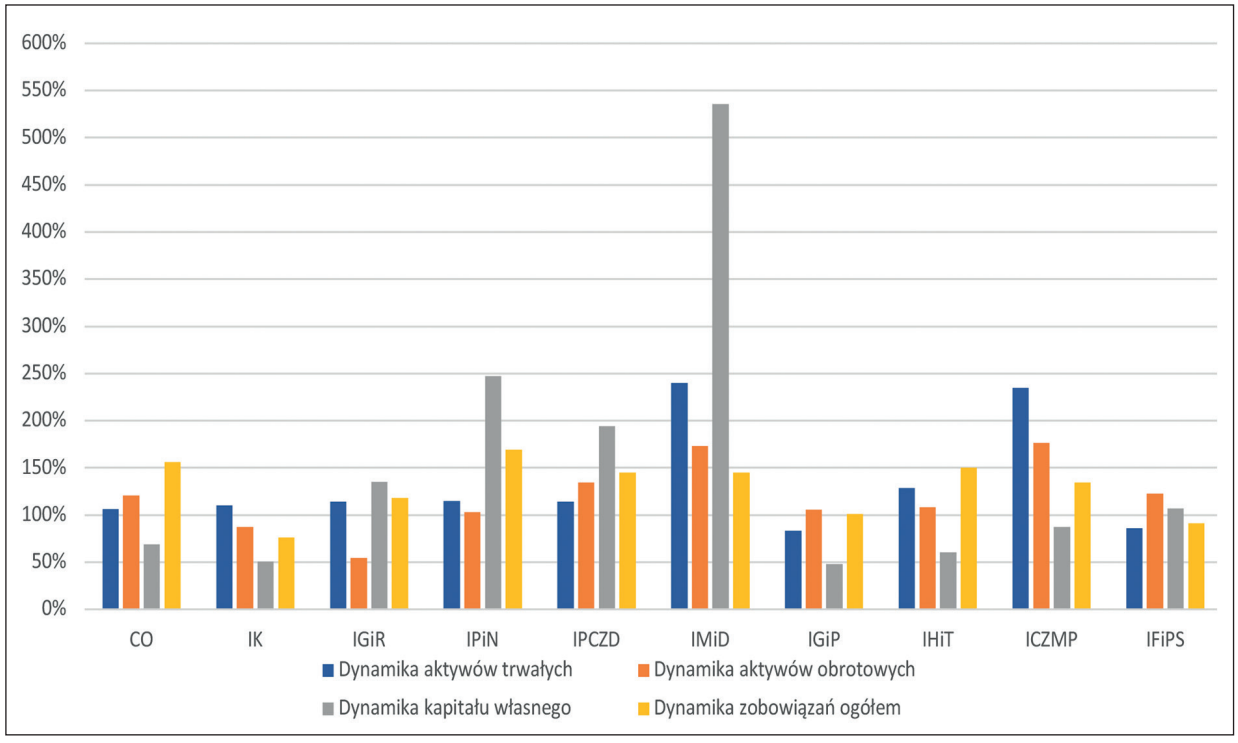
### DYNAMIKA I STRUKTURA MAJĄTKU I ŹRÓDEŁ FINANSOWANIA

W przypadku większości analizowanych instytutów wartość majątku ogółem wzrosła na przestrzeni analizowanego okresu. Średnia wartość wskaźnika dynamiki aktywów ogółem 2018/2014 wyniosła 126% (średnią obliczono poprzez zsumowanie dynamiki aktywów ogółem dla wszystkich instytutów i podzielenie tej wartości przez 10). Największy wzrost wartości majątku ogółem odnotował Instytut Matki i Dziecka, zaś spadek wystąpił w Instytucie Fizjologii i Patologii Słuchu. W przypadku pięciu instytutów wskaźnik dynamiki aktywów trwałych (2018/2014) był wyższy niż aktywów obrotowych, a wskaźnik dynamiki zobowiązań ogółem przewyższał wskaźnik dynamiki kapitału własnego również w przypadku pięciu instytutów (Ryc. 1).

**Tabela 2.** Wartości punktowe przypisane poszczególnym wskaźnikom oceny sytuacji ekonomiczno-finansowej.

| Grupa | Wskaźniki | PP |
|---|---|---|
| Wskaźnik zyskowności | Wskaźnik zyskowności netto (%) | 0-5 |
| | Wskaźnik zyskowności działalności operacyjnej (%) | 0-5 |
| | Wskaźnik zyskowności aktywów (%) | 0-5 |
| | **Razem zyskowność** | 15 |
| Wskaźnik płynności | Wskaźnik bieżącej płynności | 0-12 |
| | Wskaźnik szybkiej płynności | 0-13 |
| | **Razem płynność** | 25 |
| Wskaźnik efektywności | Wskaźnik rotacji należności (dni) | 0-3 |
| | Wskaźnik rotacji zobowiązań (dni) | 0-7 |
| | **Razem efektywność** | 10 |
| Wskaźniki zadłużenia | Wskaźnik zadłużenia aktywów [%] | 0-10 |
| | Wskaźnik wypłacalności [%] | 0-10 |
| | **Razem zadłużenie** | 20 |
| | **Razem wszystkie obszary** | 70 |

PP- przedział punktów
Źródło: Rozporządzenie Ministra Zdrowia z dnia 12 kwietnia 2017 roku w sprawie wskaźników ekonomiczno-finansowych niezbędnych do sporządzenia analizy oraz prognozy sytuacji ekonomiczno-finansowej samodzielnych zakładów opieki zdrowotnej.

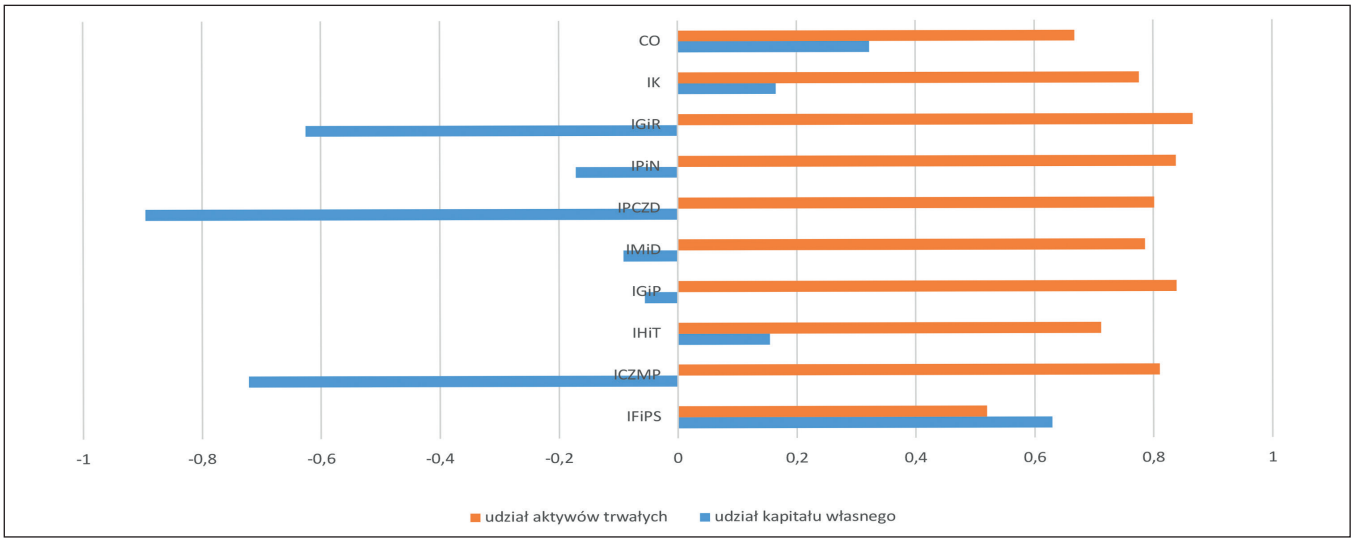



**Ryc. 1.** Dynamika aktywów trwałych i obrotowych oraz kapitału własnego i zobowiązań ogółem 2018/2014 [%].
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

Jedynym instytutem, który spełniał w latach 2014-2018 tzw. złotą regułę finansowania (majątek trwały był finansowany kapitałem własnym) w każdym roku był Instytut Fizjologii i Patologii Słuchu w Warszawie. W niektórych podmiotach udział kapitału własnego w sumie bilansowej przyjmował wartości ujemne – w 2018 roku było to sześć instytutów (Ryc. 2). Najtrudniejsza w tym aspekcie sytuacja wystąpiła w Instytucie Centrum Zdrowia Matki Polki (ujemna wartość kapitału własnego w całym analizowanym okresie).

## DYNAMIKA I STRUKTURA PRZYCHODÓW I KOSZTÓW OGÓŁEM

W analizowanym okresie (2014-2018) prawie wszystkie instytuty zwiększyły wartość swoich przychodów i kosztów




**Ryc. 2.** Udział aktywów trwałych i kapitału własnego w sumie bilansowej w 2018 r.
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

**Tabela 3.** Dynamika przychodów i kosztów ogółem w latach 2014-2018 (w %), 2014=100% .

| NIB | 2015/2014 | | 2016/2014 | | 2017/2014 | | 2018/2014 | | 2018/2014 – średnio dla wszystkich jednostek | |
|---|---|---|---|---|---|---|---|---|---|---|
| | P | K | P | K | P | K | P | K | P | K |
| IFiPS | 102 | 101 | 101 | 98 | 108 | 112 | 107 | 183 | 122 | 130 |
| ICZMP | 99 | 103 | 103 | 103 | 127 | 114 | 131 | 131 | | |
| IHiT | 113 | 113 | 114 | 114 | 122 | 125 | 129 | 139 | | |
| IGiP | 99 | 97 | 98 | 98 | 101 | 100 | 114 | 110 | | |
| IMiD | 103 | 101 | 112 | 112 | 124 | 122 | 132 | 139 | | |
| IPCZD | 104 | 105 | 110 | 110 | 119 | 114 | 129 | 119 | | |
| IPiN | 102 | 100 | 104 | 104 | 117 | 114 | 128 | 123 | | |
| IGiR | 98 | 99 | 107 | 107 | 102 | 108 | 113 | 124 | | |
| IK | 102 | 99 | 100 | 100 | 109 | 101 | 111 | 102 | | |
| CO | 103 | 108 | 108 | 122 | 118 | 132 | 130 | 133 | | |

NIB – nazwa instytutu badawczego; P- przychody; K – koszty
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

ogółem (Tab. 3). Średnio (dla całej analizowanej grupy) przychody w 2018 wzrosły o 22% w stosunku do roku 2014, zaś koszty o 30% (średnią wyliczono poprzez zsumowanie dynamiki przychodów i kosztów za 2018 rok i podzielenie tej liczby przez 10). W przypadku połowy analizowanych instytutów wartość wskaźnika dynamiki kosztów ogółem 2018/2014 przewyższała wartość dynamiki przychodów ogółem w tym samym okresie.

## WYNIK FINANSOWY I NADWYŻKA FINANSOWA

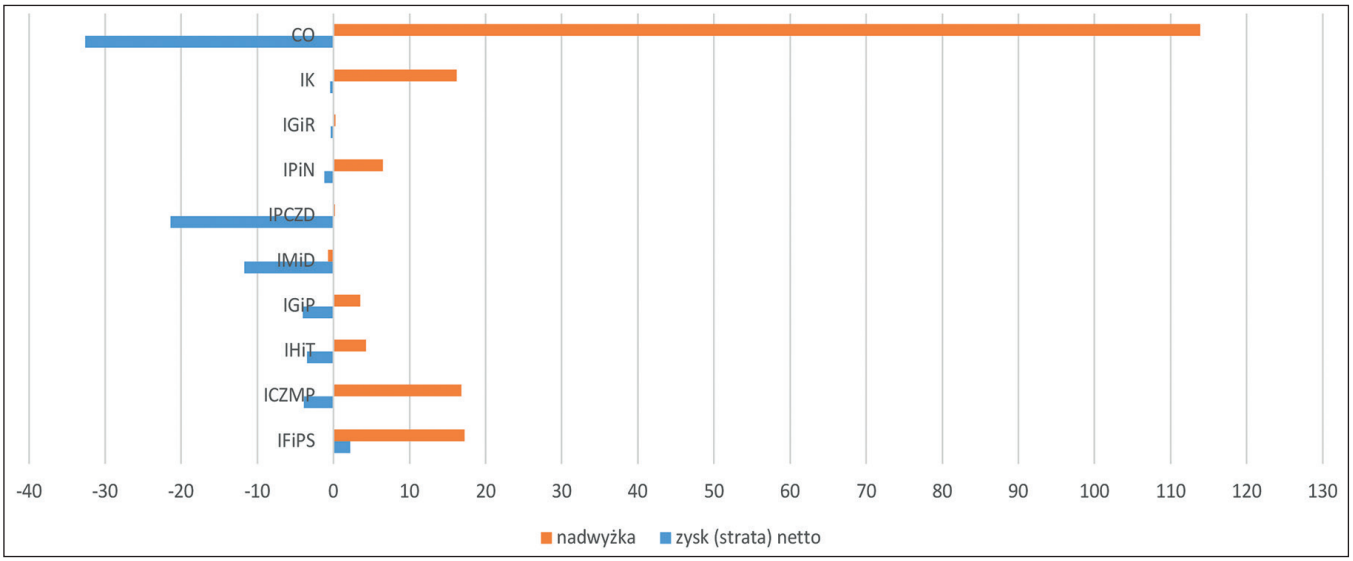
Nadwyżka finansowa definiowana jest jako suma zysku (straty) netto i amortyzacji. W 2018 roku 9 placówek generowało stratę netto (jedynie Instytut Fizjologii i Patologii Słuchu generował zysk netto). Jednakże po uwzględnieniu amortyzacji większość jednostek charakteryzowała się dodatnimi wartościami nadwyżki finansowej. Ujemną nadwyżkę wykazał jedynie Instytut Matki i Dziecka (Ryc. 3).

## STRUKTURA KOSZTÓW OPERACYJNYCH

W 2018 r. w badanej grupie instytutów największy udział w kosztach operacyjnych najczęściej zajmują wynagrodzenia (pięć placówek). W czterech jednostkach (CO, IGiR, IHiT, IFiPS) największą pozycję kosztów stanowią koszty materiałów i energii (Ryc. 4). To te dwa rodzaje kosztów we wszystkich instytutach są odpowiedzialne za ponad 50% wszystkich kosztów operacyjnych.

## ANALIZA WSKAŹNIKOWA

W Tabeli 4 zebrano wartości poszczególnych wskaźników w roku 2018. Analizowane jednostki znajdują się w bardzo




**Ryc. 3.** Poziom zysku (straty) netto i nadwyżki finansowej w roku 2018 (w mln).
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

zróżnicowanej sytuacji finansowej. Wyniki oceny punktowej, zgodnej z kryteriami przyjętymi w Rozporządzeniu z 12 kwietnia 2017 roku wskazują, że w najlepszej sytuacji finansowej był Instytut Fizjologii i Patologii Słuchu, który uzyskał najwyższe oceny punktowe wartości w obszarach: wskaźniki zadłużenia oraz płynności. Instytut ten wyróżnia się w całym analizowanym okresie największą stabilnością finansową.

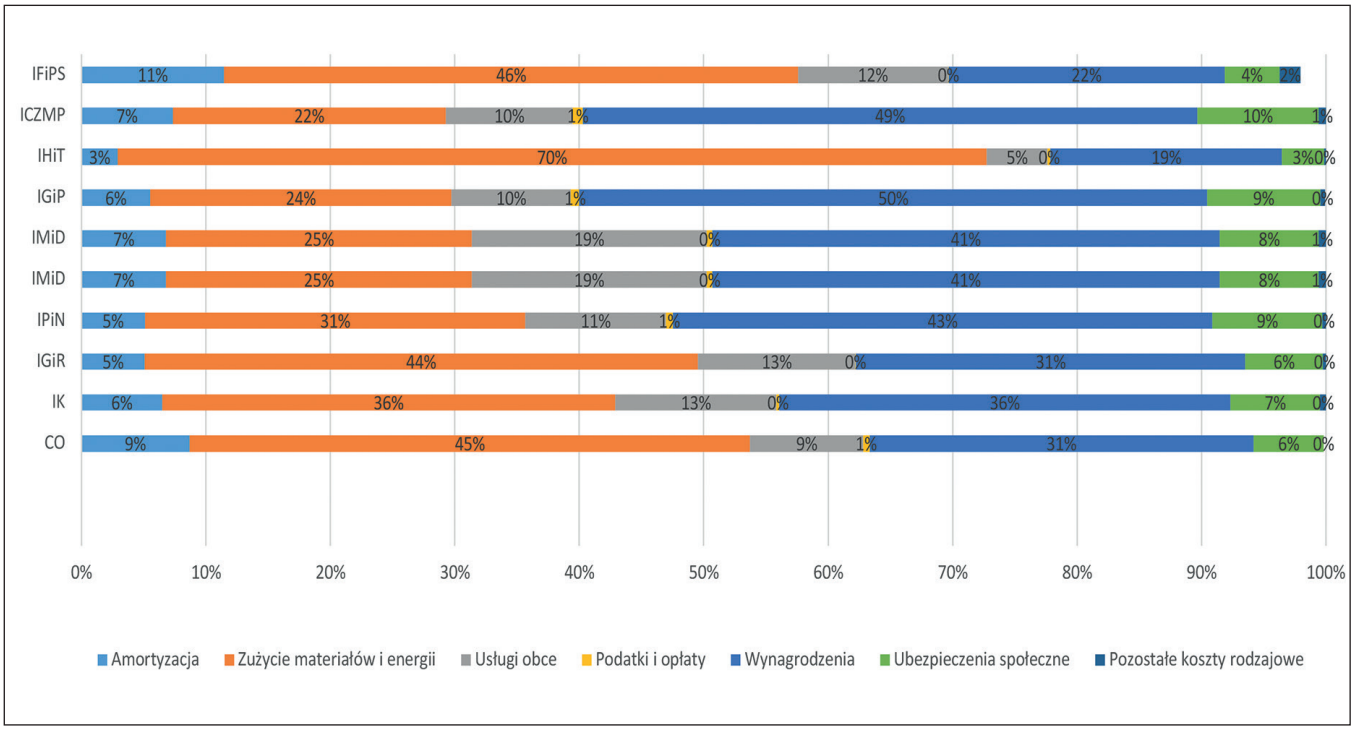
Oceny wskaźników finansowych pięciu instytutów charakteryzowały się bardzo niską wartością punktową, nierzadko poniżej 20 punktów (na 70 możliwych). Do jednostek znajdujących się w najtrudniejszej sytuacji finansowej należą Instytut Pomnik Centrum Zdrowia Dziecka oraz Centrum Zdrowia Matki Polki. Instytut – Pomnik Centrum Zdrowia Dziecka uzyskał w 2017 roku 12 punktów na 70 możliwych (17%), a w 2018 roku tylko 9 punktów (13% możliwych do uzyskania). W ostatnim analizowanym roku 9 instytutów zanotowało gorsze wyniki niż w 2017 roku co przedstawia rycina 5. Największe problemy analizowane jednostki miały z pokrywaniem swoich zobowiązań wobec wierzycieli. Niskie wartości sumarycznych wyników były spowodowane także tym, że niektóre wskaźniki przyjęły wartości ujemne z racji generowania straty netto przez analizowane jednostki.

## DYSKUSJA

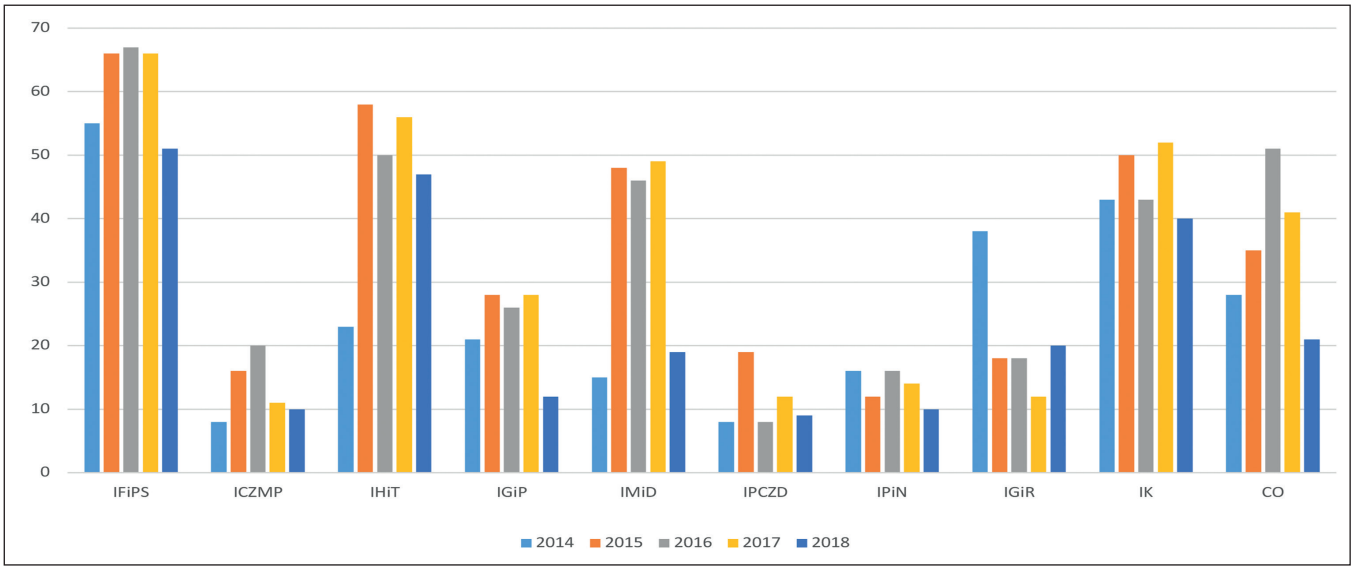
Sytuacja finansowa analizowanych instytutów badawczych jest bardzo zróżnicowana. W badanej grupie odnaleźć można zarówno jednostki, które uzyskały wysoką liczbę punktów w analizie wskaźnikowej, jak chociażby Instytut Fizjologii i Patologii Słuchu, który w latach 2014-2018 uzyskiwał ponad 70% maksymalnie możliwych do osiągnięcia punktów w każdym analizowanym roku, jak i takie, które uzyskiwały jedynie około 15% punktów (Instytut Centrum Zdrowia Matki Polki, Instytut – Pomnik Centrum Zdrowia Dziecka). Przyczyny trudnej sytuacji finansowej analizowanych podmiotów są złożone. Z jednej strony instytuty badawcze nadzorowane przez Ministra Zdrowia i świadczące usługi stacjonarne podlegają tym samym uwarunkowaniom co szpitale publiczne, z drugiej zaś realizują dodatkowe zadania i funkcje wynikające z ich szczególnej formy organizacyjno-prawnej.

Największe problemy jednostki miały ze spłacaniem swoich zobowiązań wobec wierzycieli. Problem zadłużania szpitali publicznych stanowi nieodłączną cechę polskiego systemu ochrony zdrowia od niemalże trzech dekad [7, 8]. Wśród przyczyn wyróżnia się czynniki na poziomie makro (uwarunkowania systemowe); mezo (rola organu tworzącego) i mikro (zarządzania konkretną placówką) [7]. Dostępne piśmiennictwo wskazuje jednak, że instytuty badawcze podlegle Ministrowi Zdrowia znajdowały się w roku 2018 w gorszej sytuacji finansowej niż pozostali publiczni świadczeniodawcy usług zdrowotnych (szpitale funkcjonujące w formie SPZOZ oraz spółek z całościowym /lub większościowym udziałem podmiotu publicznego) [6]. Instytuty badawcze z reguły nie generowały także zysków. Z tej racji nie otrzymywały punktów za wskaźnik zyskowności. Instytuty z małą liczbą łóżek charakteryzowały się lepszą ogólną sytuacją finansową i rzadziej generowały stratę netto (przykład: IFiPS). Żaden z analizowanych podmiotów nie wykazał stabilnego trendu poprawy sytuacji finansowej w okresie 2014-2018 definiowanej jako zwiększenie punktacji w każdym roku. Można stwierdzić, że sytuacja się pogarsza, ponieważ siedem instytutów uzyskało w roku 2018 niższy wynik oceny punktowej niż w roku 2014. Tylko w trzech przypadkach wyniki z 2018 roku był lepsze niż w 2014 roku (ICZMP, IHiT, IPCZD).

Kontrolerzy NIK w swoich raportach opublikowanych w 2012 roku i 2015 roku wśród głównych powodów złej kondycji finansowej wymienili: przyjmowanie kosztochłonnych pacjentów i przeprowadzanie wysokospecja-




**Ryc. 4.** Struktura kosztów operacyjnych w 2018 roku.
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe




**Ryc. 5.** Wyniki oceny punktowej ogólnej sytuacji finansowej instytutów badawczych w okresie 2014-2018 (maksymalna liczba punktów = 70)*.
*Dane dla wskaźników: zyskowności netto, wskaźnika rotacji należności i zobowiązań dla 2014 roku pochodziły z roku 2015, aby we wszystkich analizowanych latach możliwa była do osiągnięcia wartość punktowa wynosiła 0-70 punktów.
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

listycznych procedur medycznych, zatrudnianie wysokiej klasy specjalistów, zbyt niską dynamikę wyceny wartości punktu za świadczenia kontraktowane przez Narodowy Fundusz Zdrowia, która nie odzwierciedla rzeczywistych kosztów oraz zbyt złożoną strukturę organizacyjną, która uniemożliwia przeprowadzenie niezbędnych zmian wewnątrz placówki [14, 15]. Instytut Centrum Zdrowia Dziecka znajduje się w wyjątkowo trudnej sytuacji finansowej. Ocena funkcjonowania tego instytutu była przedmiotem niezależnych ekspertyz. W raporcie opublikowanym w 2013 roku, przygotowanym przez niezależny zespół ekspertów stwierdzono, iż poziom zadłużenia jednostki zagraża jej dalszemu funkcjonowaniu i zarekomendowano głębokie zmiany restrukturyzacyjne [16]. Wykazano także,

**Tabela 4.** Wartości wskaźników: zyskowności, płynności, efektywności, zadłużenia w 2018.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| IFiPS | 0,02 | 0,02 | 0,01 | 3,87 | 2,55 | 55,44 | 446,34 | 0,15 | 0,24 |
| ICZMP | -0,01 | -0,01 | -0,01 | 0,40 | 0,28 | 35,79 | 1028,54 | 1,23 | -1,70 |
| IHiT | -0,03 | -0,01 | -0,06 | 3,64 | 2,25 | 46,47 | 295,77 | 0,14 | 0,88 |
| IGiP | -0,03 | -0,03 | -0,03 | 0,53 | 0,32 | 58,03 | 630,99 | 0,26 | -5,65 |
| IMiD | -0,08 | -0,08 | -0,08 | 0,46 | 0,86 | 37,38 | 569,35 | 0,60 | -6,54 |
| IPCZD | -0,18 | -0,07 | -0,08 | 1,32 | 0,27 | 61,36 | 1125,79 | 1,09 | -1,46 |
| IPiN | -0,22 | -0,22 | -0,19 | 0,31 | 0,26 | 48,81 | 785,45 | 0,49 | -2,85 |
| IGiR | -0,05 | -0,07 | -0,05 | -0,03 | 0,01 | 62,29 | 1050,94 | 0,96 | 0,34 |
| IK | 0,00 | 0,00 | 0,00 | 0,98 | 0,75 | 28,14 | 363,88 | 0,23 | 1,42 |
| CO | -0,03 | -0,03 | -2,85 | -0,25 | 0,93 | 4,94 | 309,22 | 0,33 | 8,34 |

1 – wartość wskaźnika zyskowności netto, 2 – wartość wskaźnika zyskowności działalności, 3 – wartość wskaźnika zyskowności aktywów, 4 – wartość wskaźnika bieżącej płynności, 5 – wskaźnik szybkiej płynności, 6 – wskaźnik rotacji należności, 7- wskaźnik rotacji zobowiązań, 8 – wskaźnik zadłużenia aktywów, 9 – wskaźnik wypłacalności.
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia. https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe

**Tabela 5.** Wyniki oceny punktowej ogólnej sytuacji finansowej instytutów badawczych w okresie 2014-2018 (maksymalna liczba punktów = 70, 70 pkt=100%)*.

| | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Punkty | Ocena [%] | Punkty | Ocena [%] | Punkty | Ocena [%] | Punkty | Ocena [%] | Punkty | Ocena [%] |
| IFiPS | 55 | 79% | 66 | 94% | 67 | 96% | 66 | 94% | 51 | 73% |
| ICZMP | 8 | 11% | 16 | 23% | 20 | 29% | 11 | 16% | 10 | 14% |
| IHiT | 23 | 33% | 58 | 83% | 50 | 71% | 56 | 80% | 47 | 67% |
| IGiP | 21 | 30% | 28 | 40% | 26 | 37% | 28 | 40% | 12 | 17% |
| IMiD | 15 | 21% | 48 | 69% | 46 | 66% | 49 | 70% | 19 | 27% |
| IPCZD | 8 | 11% | 19 | 27% | 8 | 11% | 12 | 17% | 9 | 13% |
| IPiN | 16 | 23% | 12 | 17% | 16 | 23% | 14 | 20% | 10 | 14% |
| IGiR | 38 | 54% | 18 | 25% | 18 | 26% | 12 | 17% | 17 | 24% |
| IK | 43 | 61% | 50 | 71% | 43 | 61% | 52 | 74% | 40 | 57% |
| CO | 28 | 40% | 35 | 50% | 51 | 73% | 41 | 59% | 21 | 30% |

*Dane dla wskaźników: zyskowności netto, wskaźnika rotacji należności i zobowiązań dla 2014 roku pochodziły z roku 2015, aby we wszystkich analizowanych latach możliwa do osiągnięcia wartość punktowa wynosiła 0-70 punktów.
https://www.gov.pl/web/zdrowie/instytuty-badawcze-roczne-sprawozdania-finansowe
Źródło: opracowanie własne na podstawie danych zawartych w rocznych sprawozdaniach finansowych instytutów medycznych publikowanych na stronie Ministerstwa Zdrowia.

iż poziom wyceny procedur jest niesatysfakcjonujący, co można uznać za przesłankę generowania przez placówkę ujemnego wyniku finansowego. Z kolei w raporcie NIK z kontroli IPCZD z 2018 r., obejmującej okres 2014-2017 wskazano na następujące przyczyny złej sytuacji instytutu: brak perspektywicznych kierunków działalności naukowej, nietraktowanie działalności naukowej i prac rozwojowych jako działalności priorytetowej oraz nieprowadzenie analiz efektywności pracy pracowników placówki. W sferze finansowej plan poprawy sytuacji w latach 2015-2018 nie przyniósł oczekiwanych efektów [16, 17]. Dostępne piśmiennictwo wskazuje na występowanie zależności pomiędzy sytuacją finansową podmiotów leczniczych a jakością świadczonych usług [18] .

## WNIOSKI

Wyniki naszych analiz wskazują, że pomimo racjonalnych zaleceń NIK i różnych ekspertów dotyczących potrzeby poprawy sytuacji finansowej instytutów badawczych podległych Ministrowi Zdrowia – w okresie ostatnich pięciu lat nie nastąpiła żadna znacząca zmiana w tym obszarze. Postawić można

tezę o niskim poziomie zainteresowania głównego decydenta (Ministerstwa Zdrowia) analizowanym problemem, jak i częściową społeczną akceptacją problemu zadłużania publicznych świadczeniodawców usług zdrowotnych. W interesie pacjentów i szerzej całego społeczeństwa leży jednak ekonomiczne uzdrowienie instytutów badawczych i wszystkich podmiotów świadczących usługi medyczne, ale nie uzdrowienie polegające na kolejnym oddłużeniu, lecz takie, które wymusi na wszystkich racjonalne obchodzenie się z ograniczonymi zasobami ludzkimi, kapitałowymi i finansowymi.

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**Conflict of interest**

*Authors declare no conflict of interest*


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ORIGINAL ARTICLE

# URINARY EXCRETION OF TGF-B1 AND VEGF IN CHILDREN WITH VESICOURETERAL REFLUX

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## ABSTRACT

**The aim** of this study was to investigate the relation between urinary TGF-β1, urinary VEGF and renal scarring resulted from VUR.

**Materials and methods:** This study included 141 patients with VUR and 34 healthy sex and age matched children. The statistical analysis consisted of descriptive statistical parameters, KruskalWallis, Mann-Whitney tests and ROC analysis.

**Results:** The urine levels of TGF-β1 and VEGF were significantly increased in children with VUR, compared to the controls. The levels of TGF-β1 urine excretion in children with renal scarring were higher compared children no renal scarring. The indicators of VEGF urine excretion in children with renal scarring compared to indicators in children no renal scarring, were lower, however exceeded the indicators in children of control group. The area under the ROC curve for TGF-β1 was 109.9, for VEGF was 207.6.

**Conclusions:** The study allowed to substantiate and propose non-invasive methods for early diagnosis of renal scarring in children with VUR.

**KEY WORDS:** vesicoureteral reflux; renal scarring; TGF-β1; VEGF, children

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## INTRODUCTION

One of the most frequent form of congenital urinary tract abnormalities in children is the vesicoureteral reflux (VUR) [1]. According to International Reflux Study Committee the frequency of the nephrosclerosis development with VUR among children of the European population is 25-40% and leads to the development of a terminal stage of *chronic kidney disease* (CKD) in 40-50% of patients [2, 3]. According to modern literature, the frequency of terminal CKD in children with renal scarring (RS) constantly grows around the world [4, 5]. About 25% of children with renal scarring owing to VUR need carrying out a hemodialysis and kidney transplantation [6].

Over the past years, scientists from different countries conducted a lot of research on the analysis the renal parenchyma in children with VUR [7, 8]. Despite this, the search for new markers of renal tissue damage in patients with VUR is not stopped. Today it is known that the development of RS is a cascade *kidney remodeling processes*, which is characterized by functional and morphological changes [9].

Experimental and not numerous clinical trials demonstrated that immuneinflammatory mechanisms are involved in formation of RS with proinflammatory and profybrotic cytokines, that results in inflammation of the interstition involving tubules and blood vessels with the subsequent remodelation of the tubulointerstitial kidney tissue that causes fibrosis of a parenchyma and an atrophy of tubules [10]. Authors note that transforming growth factor β1 (TGF-β1) is a powerful profibrotic cytokine. TGF-β1 strengthens a fibroblastic proliferation and an epitelial-mezenximal transformation of endothelial cells that strengthens the fibrosis of kidney parenchyma [11, 12]. At the same time, the researchers established that podocytes synthesized vascular endothelial growth factor (VEGF) induces cellular adhesion and suppresses proliferation of fibroblasts and also supports a peritubular bloodflow, stimulates angiogenesis [13].

Despite intensive researches, the problem of noninvasive diagnostics of nephrosclerosis, especially its preclinical stages, is still being up to date.

## THE AIM

In this study we defined the level of biological markers of the fibrogenesis (TGF-β1) and angiogenesis (VEGF) in urine, depending on existence of signs of RS and also established diagnostic predictive of the level of these indicators on formation of RS in children with VUR.

## MATERIAL AND METHODS

### GENERAL INFORMATION

A prospective study was carried out from 2014 to 2019 on 141 children (38 boys and 103 girls) from 1 to 17 years of age with VUR and full clinical-laboratory remission of pyelonephritis.

The patients divided into the groups according to grade of VUR and whether or not combined with RS. The I group

**Table I.** General information on patients with VUR and pyelonephritis

| Characteristic | I group (n = 24) | II group (n = 87) | | III group (n = 30) | | Overall (n = 141) |
|---|---|---|---|---|---|---|
| | | II A (n = 69) | II B (n = 18) | III A (n = 9) | III B (n = 21) | |
| Age, (%) | | | | | | |
| 6 months – 2 years | 8 (33.3) | 27 (39.1) | 2 (11.1) | 3 (33.3) | 1 (4.8) | 41 (29.1) |
| 3 – 6 years | 6 (25.0) | 27 (39.1) | 2 (11.1) | 2 (22.2) | 6 (28.6) | 43 (30.5) |
| 7 - 11 years | 6 (25.0) | 10 (14.5) | 10 (55.6) | 3 (33.3) | 7 (33.3) | 36 (25.5) |
| 12 - 17 years | 4 (16.7) | 5 (7.3) | 4 (22.2) | 1 (11.1) | 7 (33.3) | 21 (14.9) |
| Male/Female | 4/20 | 13/56 | 7/11 | 4/5 | 11/10 | 39/102 |
| Index urinary tract infection, no. (%) | 20 (83.3) | 23 (33.3) | - | 2 (22.2) | - | 45 (31.9) |
| The incidental course of pyelonephritis Recurrent pyelonephritis | 4 (16.7) | 46 (66.7) | 18 (100.0) | 7 (77.8) | 21 (100.0) | 96 (68.1) |
| Bilateral VUR, no./total no. (%) | 7/24 (29.2) | 15/69 (21.7) | 3/18 (16.7) | 2/9 (22.2) | 7/21 (33.3) | 34/141 (24.1) |

VUR - vesicoureteral reflux; no - absolute number;

**Table II.** Laboratory data in children with VUR

| Characteristic | I group (n = 24) | II group | | III group | |
|---|---|---|---|---|---|
| | | II A (n = 69) | II B (n = 18) | III A (n = 9) | III B (n = 21) |
| Hemoglobin, g/l* | 126 (116; 134) | 117 (111; 129) | 124 (120; 131) | 120 (107; 122) | 116 (108; 124) |
| | MW test: $p_{I-II} = 0.2429$; $p_{II-III} = 0.6782$; $p_{I-III} = 0.1917$; | | | | |
| *ESR*, mm/h* | 5.5 (4.0; 15,5) | 5.0 (3.0; 11.0) | 7.0 (4.0; 10.0) | 5.0 (3.0; 7.0) | 7.0 (4.0; 11.0) |
| | MW test: $p_{I-II} = 0.6441$; $p_{II-III} = 0.9155$; $p_{I-III} = 0.7873$; | | | | |
| Serum creatinine, μmol/l* | 67.6 (58.7; 86.0) | 68.0 (57.4; 90.0) | 72.7 (60.4; 83.9) | 63.6 (53.1; 68.3) | 100.0 (61.9; 140.0) |
| | MW test: $p_{I-II} = 0.6804$; $p_{II-III} = 0.1094$; $p_{I-III} = 0.1717$; | | | | |
| GFR, mL/min/1.73 $m^{2}$ * | 80.0 (56.0; 96.0) | 80.0 (61.0; 95.0) | 85.0 82.0; 108.0) | 89.0 (68.0; 92.0) | 73,0 (51.0; 94.0) |
| | MW test: $p_{I-II} = 0.5007$; $p_{II-III} = 0.2006$; $p_{I-III} = 0.6198$; | | | | |

VUR - vesicoureteral reflux; ESR - *erythrocyte sedimentation rate*; GFR - glomerular filtration rate; MW - Mann Whitney for paired comparison; Data are presented in the form Me (Lq; Uq)

comprised 24 children had I grade VUR, no RS; the II group comprised 87 patients had II and III grade VUR: among them 69 children no RS (IIA group), 18 – with RS (IIB group), the III group comprised 30 patients had V grade VUR: among them 9 children no RS (IIIA group), 21 patients had RS (IIIB group). The control group included 31 healthy children (19 girls and 12 boys) of similar age that had no complaints, clinical signs, data anamnesis which would confirm presence of any chronic disease or congenital defects of urinary system organs or an acute disease.

Complex assessment of the patient condition presupposed studying of the anamnesis, full objective inspections and laboratory tool researches for verification of the diagnosis by KDIGO 2012 recommendations [14]. VUR was diagnosed according to the voiding cystourethrography and graded according to International Study Classification [15, 16].

Renal scarring was diagnosed with dimercaptosuccinic acid (DMSA). It was noted that DMSA scan was performed 3-4 months after exacerbation period of pyelonephritis.

TGF-β1 and VEGF urinary excretion levels were analyzed with the ELISA (enzyme-linked immunosorbent assay). TGF-β1 levels were determined with the help of standard sets (Catalog No: BMS249/4 and BMS249/4TEN human TGF-beta1, Bender MedSystems GmbH, Austria), VEGF levels – (Catalog No: BMS277/2 and BMS277/2TEN human VEGF-A, Bender MedSystems GmbH, Austria) according to manufacturers' instructions.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of the Kharkiv National Medical University, Ukraine. Information consents were signed by all participants and/or their parents gave written informed consent (Protocol No.8 dated October 3, 2018). Research was conducted in accordance with the World Medical Association's Helsinki Declaration.

**Table III.** Urine excretion of TGF-β1 and VEGF in children with VUR

| Group | Parameter | |
|---|---|---|
| | TGF-β1, pg/ml | VEGF, pg/ml |
| I group (n = 24) | 77.5* (26.7; 140.1) | 206.6* (126.5; 265.7) |
| II A group (n = 69) | 70.2* (28.7; 105.5) | 222.0* (150.6; 358.6) |
| II B group (n = 18) | 139.5* (117.2; 215.9) | 178.4* (137.3; 203.2) |
| III A group (n = 9) | 30.7* (29.1; 40.5) | 244.2* (189.3; 295.8) |
| III B group (n = 21) | 108.6* (56.2; 133.5) | 124.9* (104.9; 152.6) |
| Controls group (n = 31) | 14.0 (3.1; 29.2) | 40.9 (27.2; 59.3) |

*p<0.05 in comparison with control group; TGF-β1 - transforming growth factor-beta1; VEGF - vascular endothelial growth factor; Data are presented in the form Me (Lq; Uq)

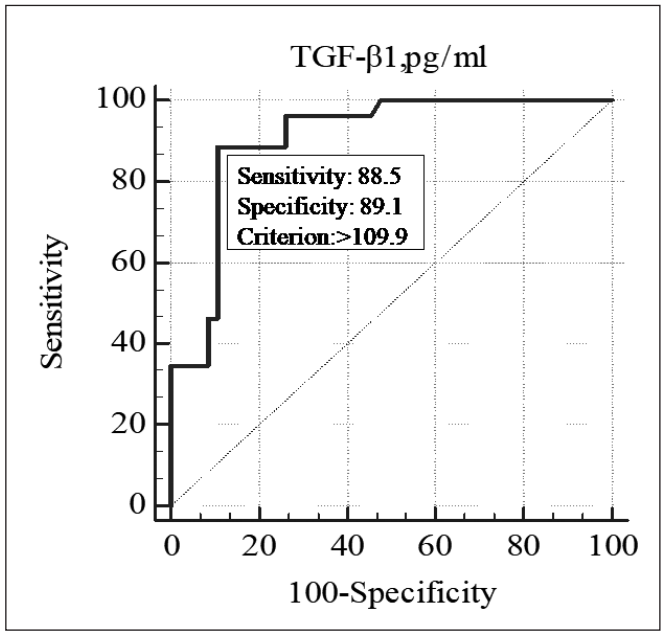


**Fig.1.** Receiver operating characteristic (ROC) curve of *TGF-β1* for the prediction of renal scarring in children with VUR.

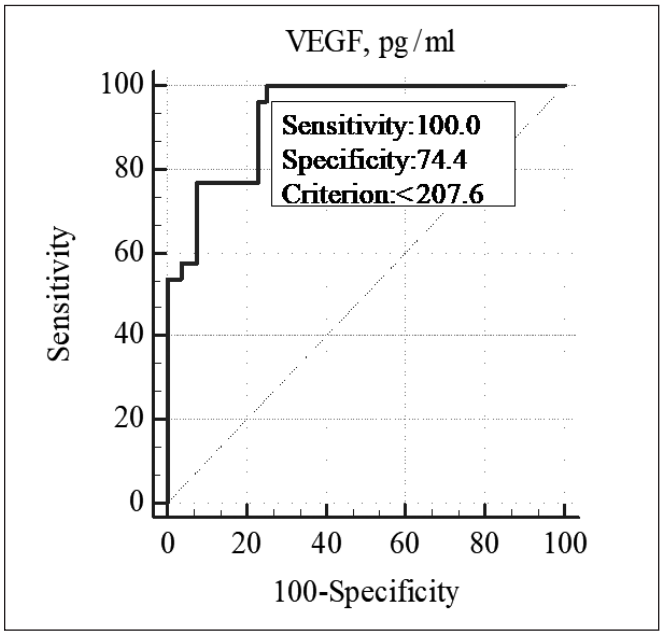


**Fig.2.** Receiver operating characteristic (ROC) curve of VEGF for the prediction of renal scarring in children with VUR.

## STATISTICAL ANALYSES

Statistical analysis was performed using «STATISTICA 7». Median (interquartile range IQR) values and frequencies are provided for the description of continuous and categorical variables, respectively. The Kruskal-Wallis test was used for intergroup comparisons. The MannWhitney U test was performed to compare differences between two independent groups.

Receiver operating characteristic (ROC) curves were drawn for variables to determine the optimal «cut-off» values to predict an endpoint. Statistical «cut-off» values were calculated by minimizing the distance between the point with specificity=1 and sensitivity=1 and various points on the ROC curve. The areas under the ROC curve for urinary TGF-beta1 (pg/ml) and VEGF (pg/ml) were calculated. The sensitivity, specificity and predictive values were calculated in comparison to the control group. The results were evaluated in the confidence interval of 95%, and significance was evaluated at the level of p<0.05.

## RESULTS

There were 141 children aged 1 to 17 years with VUR and full clinical-laboratory remission of pyelonephritis. Analysis of the age characteristics of the children of the main group showed that 41 children (29.1%) were aged 6 months to 2 years, 43 children (30.5%) from 3 to 6 years, 36 children (25.5%) from 7 to 11 years and 21 of patients (14.9%) at the age from 12 to 17 years. 102 (72.3%, p<0.05) of the children were girls (Table I).

The results of the voiding cystourethrography demonstrated more frequent high grade VUR (III, IV and V) in surveyed patients than lowgrade VUR (I, II) (82.9% and 17.1%, p<0.05, respectively). At all grades of VUR, unilateral affect is probably observed more often than bilateral (all p<0.05). The average age of the disclosure of the disease was 4.1 ± 2.1 years.

In 39 (27.7%) of the examined children were found signs of RS. In patients of I group no signs of RS were found. Depending on a reflux grade, the specific weight of patients

with RS, particularly in 18 (20.7%) children of II group and 21(70.0%) children of III group increases ($p<0.05$). At the high grade of VUR the risk of RS is higher, than in grades I-II (RR = 8.00, 95% CI [1.16; 55.44]).

There were no statistically significant differences between the groups in hemoglobin, erythrocyte sedimentation rate, serum creatinine. Meanwhile, with increasing grading of reflux, the amount of glomerular filtration rate decreased, but the changes were not statistically significant ($p>0.05$) (Table II).

Patients in all groups had significantly higher urinary TGF-β1 level when compared to control group (KW H=30.72, p=0.0001, MW $p_{cI}$=0.0078; $p_{cIIA}$=0.0000; $p_{cIIB}$=0.0002; $p_{cIIIA}$=0.0047; $p_{cIIIB}$=0.0001). However, TGF-β1 in patients showed increasing trend that became statistically significant in children with VUR and RS when compared to children with VUR no RS (MW $p_{IIAIIB}$=0.0067, $p_{IIIAIIIB}$=0.0057) (Table III).

Patients in all groups had significantly higher urinary VEGF level when compared to control group (KW H=43.134, p=0.0003, $p_{cI}$=0.0003; $p_{cIIA}$=0.0000; $p_{cIIB}$=0.0000; $p_{cIIIA}$=0.0022; $p_{cIIIB}$=0.0003). However, VEGF in patients showed decreasing trend that became statistically significant in children with VUR and RS when compared to children with VUR no RS, but exceeded data of indicators in children of control group (MW $p_{IIAIIB}$=0.0354, $p_{IIIAIIIB}$=0.0101, $p_{cIIB}$=0.0000, $p_{cIIIB}$=0.0003).

The obtained data became the reason for the determination of the urine VEGF by logistic regression using the ROC-analysis method, with the determination of the specificity and sensitivity of the method with the subsequent prediction of RS in children with VUR.

The obtained data became the reason for the determination of the urine TGFβ1 and VEGF by logistic regression using the ROC-analysis method, with the determination of the specificity and sensitivity of the method and subsequent prediction of RS in children with VUR. The area under the ROC curve (AUC) for TGF-β1 was 0.906. The optimal «cut-off» for TGF-β1 was >109.9 pg/ml (sensitivity, 88.5 %; specificity, 89.1 %) (Fig. 1). The area under the ROC curve (AUC) for VEGF was 0.932. The optimal «cut-off» for VEGF was <207.6 pg/ml (sensitivity, 100.0 %; specificity, 74.4 %) (Fig. 2).

## DISCUSSION

In our study, the age group of under 6 children was prevailed (59.6%, $p<0.05$) that corresponds to statistics on age distribution of patients with VUR [17, 18]. At distribution for sex, irrespective of age in all groups of the examined children the girls prevailed over the boys ($p<0.05$). Similar to results of other published studies in the literature [19].

According to M. Życzkowski et al., there is an increased risk of permanent kidney damage, especially at high grade of VUR [20]. Scientists of the different countries reported, that children with grade III or IV, V reflux are more likely to have larger RS [21, 22]. We established that risks of emergence of RS in children with high grade of VUR (III, IV, V) are eight times higher, than children with VUR of I-II grades. In accordance with the literature, this has confirmed the hypothesis that as VUR stage increases, the risk for RS also increases.

In our study, urinary TGF-β1 level was found to be increased in patients with VUR compared to the control group. Urinary TGF-β1 levels in children with RS ware significantly higher compared to patients no RS. Merrikhi A. showed a relationship between urin TGF-β1 and RS and emphasized the importance of this indicator [23]. Silva A. et al. reported that, urinary levels of TGF-β1 were significantly higher in patients with reduced DMSA uptake on technetium-99m DMSA scintigraphy [24]. In the context of its known profibrotic effects, these findings suggest that TGF-β1 contributes to chronic tubulointerstitial fibrosis.

The results of present study show that urinary VEGF levels were higher in all patient with VUR compared to health children. Indicators of urine excretion of VEGF in children with RS, compared to indicators of patients no RS, were significantly higher, however exceeded indicators in children of control group that can point to remodeling of a kidney blood-flow even in patients no RS. Avgustin N. et al. noted a role of VEGF as early predictor of progressing of CKD in patients with glomerular diseases and defined that increase in VEGF levels provides progressing of renal scarring [27].

The conducted ROC-analysis allowed to determine the "cut-off" value of TGF-β1 and VEGF in which the most likely development of renal scaring in children with VUR is still in the onset of the disease.

## CONCLUSIONS

Our study suggests that the established urine excretion level of fibrogenesis and angiogenesis, can be used as non-invasive test for monitoring RS associated with VUR. That will help to prevent the development RS, to define tactics of treatment and observation of patients with VUR.

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**Conflict of interest:**
*The Authors declare no conflict of interest.*

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ORIGINAL ARTICLE

# THE ROLE OF SECRETING FUNCTION OF DECIDUA IN THE DEVELOPMENT OF COMPLICATIONS OF GESTATION PROCESS IN PREGNANT WOMEN WITH A PAST HISTORY OF CHRONIC ENDOMETRITIS

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## ABSTRACT

**The aim:** To determine the serum FAMG in the I and II trimester of pregnancy in women with a past history of chronic endometritis, and to clarify its impact on the development of pathology of pregnancy.

**Materials and methods:** The level of FAMG was determined at 6-8 and 16-18 weeks of gestation in 135 pregnant women with a past history of chronic endometritis, who received treatment of chronic endometritis at the stage of pregravid preparation and 168 women who became pregnant without its prior treatment. The dependence of the development of pre-eclampsia on the level of FAMG at the early stages of pregnancy has been evaluated.

**Results:** At 6-8 weeks of pregnancy, the level of FAMG in women with a past history of chronic endometritis was 20.6% lower (122.4 ± 7.6 ng/ml) compared to the control group. In FAMG of 90.3 ± 4.3 ng/ml at 6-8 weeks of gestation, spontaneous abortion occurred in 100% of cases within the next 2 weeks. FAMG lower than 122,1 ± 3,0 ng/ml can be the predisposing factor for the development of pre-eclampsia.

**Conclusions:** Reduced FAMG in the beginning of pregnancy in women with untreated chronic endometritis in the past history increases the incidence of miscarriages at the early stages by 2.6 times, and by 1.8 times the probability of preeclampsia development. Treatment of chronic endometritis at the stage of pregravid preparation promotes the increase of FAMG by 24,6% compared to untreated women that reduces the probability of complications during the subsequent course of pregnancy.

**KEY WORDS:** chronic endometritis, pre-eclampsia, fertility α2-microglobulin, habitual noncarrying of pregnancy

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## INTRODUCTION

Prevention of preeclampsia is crucial in management of pregnancy, since this pathology causes perinatal and maternal morbidity and mortality not only in Ukraine but also worldwide [1]. This issue is especially important in the context of the ineffectiveness of any treatment for this pathology, since its occurrence is due to pregnancy itself and is formed much earlier than the onset of the first symptoms [2]. Pre-eclampsia (PE), like most placental pathologies, is associated with the disturbances in the formation of utero-placental blood flow at the stage of trophoblast invasion into the spiral arteries of the uterus [2,3]. Adequate endovascular migration of trophoblast leads to the destruction of the smooth muscles of the spiral arteries, leading to substitution with the fibrinoid masses, and, consequently, the minute vessels of the uterus transform into wide channels with low resistance and relative tolerance to the action of vasopressor factors [2,3]. This is how the massive and stable utero-placental blood flow is formed. The study of placental bed and uterine wall biopsy after hysterectomy showed that in the course of physiological pregnancy, up to 95% of the spiral arteries of the uterus, participating in the formation of the placenta, are subject to trophoblastic changes [3]. At the same time, with the development of pre-eclampsia, only 40% of arteries are transformed [3]. Unchanged vessels retain the endothelial layer, which is the basis for the development of endothelial dysfunction, vasoconstrictions [2] and impaired blood flow in the uterine and spiral arteries in pre-eclampsia [4].

Thus, the development of pre-eclampsia occurs in the beginning of pregnancy and in this process the interaction of three components is crucial: fetal (trophoblast), maternal (endometrium, transformed into the uterine decidua, spiral vessels), and immunological (factors that determine the tolerance of the mother to the "fetal transplant" and regulate the process of both interstitial and endovascular invasion of the trophoblast) [2,3]. In the regulation of the trophoblast invasion the maternal aspect is ensured by the ability of the decidual membrane to synthesize fertility α2-microglobulin (FAMG) [5]. FAMG (glycodelin-A) is a glycoprotein secreted by the glands of the endometrium and the decidual membrane [5,6]. It is a powerful stimulator for the synthesis of monocytes and macrophages of the Interleukin-6 [5], which plays an important role in the trophoblast invasion, since it increases the activity of matrix metalloproteinases, induces chemotaxis and cellular migration [5,6]. On the other hand, the inadequate development of FAMG (for example, concomitant with

chronic intrauterine infection in chronic endometritis (CE) in the past history before pregnancy [7,8]) may be one of the mechanisms that limit the invasive capacity of trophoblast and lead to pathology of pregnancy associated with the formation of the placenta at the early stages (and prior pre-eclampsia).

## THE AIM

The paper was aimed at determining the serum FAMG in the I and II trimester of pregnancy in women with a past history of chronic endometritis and to clarify its impact on the development of pathology of pregnancy.

## MATERIALS AND METHODS

A comprehensive clinical-laboratory and instrumental study of women, who planned pregnancy, became pregnant and gave birth to children in medical facilities of Poltava city and Poltava region from 2010 to 2018 was carried out. The administration of the medical facilities and the subjects were fully informed about the purpose of our research and signed the written consent form. All studies performed during the course of work were approved by the Ethics and Biomedical Ethics Commission of the Ukrainian Medical Stomatological Academy and were conducted in accordance with the Rules of Humane Treatment of Patients in compliance with the requirements of the Tokyo Declaration of the World Medical Association, the International Recommendations of the Helsinki Declaration on Human Rights, the Convection of the Council of Europe human rights and biomedicine, Laws of Ukraine, Orders of the Ministry of Health of Ukraine and the requirements of the Ethical Code of Ukrainian Doctor.

20 healthy pregnant women with unremarkable medical history (control group) and 303 pregnant women who had histologically confirmed signs of CE diagnosed before pregnancy (main group) were examined. Among women of the main group 135 women received treatment of CE at the stage of pregravid preparation according to the developed regiment (Group I). The latter included antibacterial, anti-inflammatory, antiviral and metabolic drugs, as well as progesterone (in hypertrophic forms of CE) or femostone 2/10 (in case of atrophic CE) during 3 months. The choice of antibacterial drug was made taking into account the pathogen, and in the event that it was not detected, 500 mg azithromycin was used daily from the beginning of menstruation for 3 days with subsequent repeated course during the next menstruation at the same dosage. Other 168 pregnant women of the main group refused from the treatment of CE during the pregravid preparation (Group II), which was documented in writing.

Since chronic endometriosis, diagnosed before pregnancy, can develop pre-eclampsia, we monitored the course of pregnancy to detect presence or absence of signs of this complication. In addition, at 6-8 and 16-18 weeks of pregnancy, a blood test was made to determine the serum FAMG. Upon gestation, patients in both groups were retrospectively divided into subgroups depending on the presence and severity of PE: "no pre-eclampsia" subgroup, "moderate pre-eclampsia" subgroup and "severe pre-eclampsia" subgroup. In order to find out whether the change of FAMG values in the beginning of pregnancy is associated with the manifestations of PE in later periods of the gestational process, the serum FAMG at 6-8 and 16-18 weeks of pregnancy was retrospectively evaluated in women of these subgroups, using the "Fertitest-M" test system. The method is based on the principle of enzyme-linked immunosorbent assay, which includes the immunological response of the antigen-antibody on the sandwich principle and the enzymatic reaction, which leads to the development of color changes of the solution of chromogen depending on the concentration of antigen (sandwich ELISA).

## RESULTS

At 6-8 weeks of pregnancy, serum FAMG in women, who did not receive pregravid treatment of CE was 122.4 ± 7.6 ng/ml, which was significantly lower the control values for this period (154.0 ± 7.1 ng/ml, ($p<0.05$)). In women who became pregnant after treatment of CE, the level of FAMG (152.5 ± 7.3 ng/ml) exceeds the value in the group of untreated women before pregnancy by 24.6%, approximating to the reference values ($p<0,05$)).

With the development of pregnancy, the amount of blood FAMG decreases, accounting for 96.4 ± 4.2 ng/ml at 16-18 weeks of pregnancy in women of the control group, which was 37.5% lower the values recorded at 6-8 weeks of pregnancy. At this term no significant difference compared to FAMG values in the control group was noted in women of Group I and Group II (93.2 ± 7.1 ng/ml and 85.0 ± 9.6 ng/ml, respectively, $p> 0.05$).

Noteworthy, 4 women of Group I (2.96%) and 23 women of Group II (13.7%) had miscarriage before 8 weeks of pregnancy. The serum FAMG in women with spontaneous abortion before 8 weeks of gestation was an average of 90.3 ± 6.9 ng/ml, which was extremely low for this term of pregnancy (58.6% lower the control values of 154.0 ± 7.1 ng/ml ($p <0.05$)).

At 8-12 weeks of pregnancy, 8 women of Group I (5.9%) and 16 women of Group II (9.5%) experienced spontaneous abortion. Between 12 and 22 weeks of pregnancy, 2 more women of Group I (1.48%) and 2 women in Group II (1.2%) had pregnancy loss. We have calculated the level of FAMG in women with pregnancy loss before 16-18 weeks of gestation, recorded at 6-8 weeks' gestation period. It was 28% lower than the control values and was 111.4 ± 6.6 ng/ml ($p <0.05$).

After 22 weeks of pregnancy, 1 woman (0.74%) of Group I and 2 women (1.2%) in Group II experienced premature labor. The examined women of Group I (n=2 (1.5%)) and Group II (n=15 (8.9%)), who required premature delivery due to obstetric pathology, gave birth to preterm babies. The causes of premature delivery were fetal distress (in this case, cesarean section was performed for one woman

(0.74%) of Group I and 6 women (3.6%) of Group II) before 36 weeks of pregnancy), intrauterine growth restriction associated with placental dysfunction and blood flow disorder in the utero-placental complex (3 women (1.78%) in Group II) and severe pre-eclampsia. Severe PE was the cause for premature delivery of 6 women (3.4%) in Group II and 1 woman (0.74%) in Group I.

Generally, PE complicated the course of pregnancy in 37 out of 116 women in Group II, who preserved their pregnancy to the third trimester of pregnancy (32.1%). In the group of women treated before pregnancy, this index was 17% (20 women out of 119 patients in Group I who preserved pregnancy to the third trimester of pregnancy).

Notably, moderate manifestations of PE were noted in women of Group I in 85% of cases (17 women), and severe PE was diagnosed only in 15% (3 women). Among women of Group II, severe PE occurred in 27% of cases (10 out of 37 women), and moderate manifestations of PE were noted in 73%. The average term when PE arose was 32.3 ± 0.4 weeks of pregnancy in Group I and 29.4 ± 0.6 weeks of pregnancy in Group II. Premature delivery, as mentioned above, required 1 woman from Group I and 6 women from Group II, accounting for 5% and 16.2%, respectively, for all PE women.

In women of both groups, whose pregnancy was complicated with moderate and severe PE, we calculated the serum FAMG values recorded at 6-8 weeks and 16-18 weeks of gestation. It has been found that FAMG value was 156.8 ± 7.0 ng/ml in women of Group I without developed PE at 6-8 weeks of pregnancy (almost no difference from the control values; p>0,05); in women who developed moderate PE the FAMG value was 131.9±3.9 ng/ml, which was 15% lower the control values and significantly different from them and from values in the subgroup without preeclampsia (p <0.05). In women of Group I, who developed severe pre-eclampsia, the beginning of pregnancy was accompanied by lower serum FAMG: 126.3 ± 1.5 ng/ml, which was 18% lower the values of the control group (p<0.05) and 19,5% lower the values of pregnant women without pre-eclampsia (p <0,05) and 4.3% lower the values of pregnant women with moderate pre-eclampsia (p> 0,05).

At 16-18 weeks of pregnancy, women of Group I who did not develop PE, FAMG was 95.8 ± 3.6 ng/ml, which was almost similar to reference values for this period (p> 0.05). In pregnant women, who developed moderate and severe PE, FAMG was 80.3 ± 4.8 ng/ml and 77.8 ± 1.5 ng/ml, respectively, that was 16,8% and 19,4%, respectively, less than the control values and differ significantly from them and from the values of the subgroup without pre-eclampsia (p<0,05).

The content of blood plasma FAMG in women, who did not receive treatment of CE during the pregravid preparation (Group II), had the same pattern. However, the reduction of FAMG in relation to the required values for corresponding term of pregnancy was even more pronounced in them. At 6-8 weeks of pregnancy the FAMG value in the subgroup of those women who developed moderate and severe PE was 20.8% (122.1 ± 3.2 ng/ml (p <0.05)) and 26,6% (113,1 ± 3,0 ng/ml; (p <0,05)), respectively that was lower than the control values. At 16-18 weeks of pregnancy, the decrease in FAMG relative to the control values (96.4 ± 4.2 ng/ml) was 24.9% in the subgroup of women with subsequent development of moderate PE (72.4 ± 2.5 ng/ml; p<0.05) and 31.3% in the subgroup of women with severe PE (66.2 ± 2.7 ng/ml; p<0.05).

## DISCUSSION

The study demonstrated that the presence of CE in the past history impacted significantly the progress of the gestational process in women who became pregnant with such complication. This is especially true for the habitual noncarrying of pregnancy. We have stated that pregnancy loss in women with a past history of CE (without pregravid preparation) was approximately 34.5%, with the majority of them occurring at early stages of pregnancy, up to 8 weeks of gestation (the share of all miscarriages was 56.1%).

We consider this to be related to endometrial changes in chronic infections, which are accompanied by a decrease in the secretory capacity of its glands to the synthesis of FAMG and limit its implantation potential (invasive trophoblast ability decreases, implantation is impaired, which creates preconditions for abortion [3,6]). It has been proved by the works of numerous investigators who believe that the reduction of FAMG in CE is one of the prerequisites for the development of infertility in this pathology [9,10]. The findings of our research show that even at the onset of pregnancy with untreated CE, the level of FAMG is significantly lower (by 20.6%) than the control values and is associated with a higher incidence of abortion. Thus, FAMG values in women who lost their pregnancy during the I and II trimesters at 6-8 weeks of pregnancy was 35.4% lower, and those patients whose values did not exceed 90.3 ± 4.3 ng/ml within the next two weeks lost pregnancies in 100% of cases.

On the contrary, in women who received treatment of CE during pregravid preparation with the use of antibacterial and hormonal medications the level of FAMG at 6-8 weeks of pregnancy was by 24.6% higher than the FAMG level in untreated women, approximating to the control values for this period. Admittedly, the total share of miscarriages is also lower not only before 8 weeks of pregnancy (the percentage of reproductive losses in women who received treatment before pregnancy at this term is by 4.6 times lower than in women with untreated CE in the past history), but also between 8 and 12 weeks of pregnancy. Thus, in women who did not receive pregravid treatment of CE, spontaneous abortions at 8 to 12 weeks of pregnancy occurred in 9.5% of cases compared to only 5.9% in treated women. We hypothesize that reduction in the percentage of pregnancy loss by 1.6 times in this period in treated women is likely to be explained by more successful luteoplacental transition, ensured by the maintenance of the luteal phase of the menstrual cycle during pregravid preparation.

In addition to a significant share of spontaneous abortion, we have established a high rate of premature delivery, which in women with CE was mostly due to intrauterine growth restriction and fetal distress, as well as pre-eclampsia. The probable cause for these complications, including pre-eclampsia, is the impaired gestational transformation of the spiral arteries during the I and II wave of trophoblast cell invasion, which results in the disorder of normal blood flow formation in the utero-placental complex [2,4]. The deficiency of FAMG in women with CE in the past history [8] plays an important role in these processes due to reduced invasive capacity of the trophoblast [5]. This is confirmed by the findings of our study, indicating that the low level of FAMG in the beginning of pregnancy in women with CE in the past history is associated with a high incidence of subsequent pre-eclampsia development and its more severe course. Thus, the incidence of pre-eclampsia in women with CE in the past history was 32.1%, and in one third of cases it occurred in severe form and had an early manifestation (29.4 ± 0.6 weeks of pregnancy). In fact, the level of FAMG at 6-8 weeks of pregnancy in women whose pregnancy was subsequently complicated by the development of pre-eclampsia was on the average by 17% lower as compared to patients whose pregnancy was developing without pre-eclampsia. In women with severe manifestations of pre-eclampsia, serum FAMG at 6-8 weeks of pregnancy did not exceed the values of 113.1 ± 3.0 ng/ml.

Moreover, treatment of CE at the stage of pregravid preparation promote prevention of pre-eclampsia, since, the findings showed, that the incidence of pre-eclampsia and the probability of its severe manifestations in women after treatment is reduced by 1.8 times. At 6-8 and 16-18 weeks of pregnancy, women who received treatment had higher levels of FAMG (15.9% and 9.6%, respectively), which is likely to explain the protective effect of such treatment on the course of pregnancy and its ability to prevent the development of preeclampsia.

## CONCLUSIONS

1. Untreated chronic endometritis, which occurred before gestation, increases the probability of miscarriage by 2.6 times at the early stages, as well as by 1.8 times the probability of preeclampsia development.
2. Reduced level of FAMG in the beginning of pregnancy is likely to be a prerequisite for the formation of complications of gestational process:
   - serum FAMG in women who became pregnant with CE in the past history and whose pregnancy was terminated before 8 weeks of gestation was 58.7% lower than the control values;
   - in women with untreated CE in the past history, who subsequently developed pre-eclampsia, the level of FAMG was by 23.0% lower than in the control group at 6-8 weeks of gestation.
3. Critical level of FAMG up to 90.3 ± 4.3 ng/ml at 6-8 weeks of pregnancy caused miscarriage in 100% of cases within the next 2 weeks.
4. The level of FAMG lower than 122,1 ± 3,0 ng/ml is considered the predisposing factor for the development of preeclampsia during the course of pregnancy.
5. Treatment of CE at the stage of pregravid preparation promotes the increase of the serum FAMG by 24,6%, by 4,6 times reduces the probability of spontaneous abortion at early stages, as well as by 1,8 times prevents the development of pre-eclampsia, especially its severe forms.

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ORIGINAL ARTICLE

# CURRENT ISSUES ON PROVISION OF SERVICES TO WOMEN DURING PREGNANCY AND POSTPARTUM PERIOD BY FAMILY DOCTORS

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## ABSTRACT

**The aim** is to determine the types of medical services that family doctors should provide to women during pregnancy and in the postpartum period and the competencies they should have to provide these services and the most acceptable ways to acquire them.

**Materials and methods:** During the study, the method of expert assessment, statistical method and method of structural and logical analysis were used. 50 obstetricians and gynaecologists who provide women with outpatient care and 50 family doctors acted as independent experts in the study.

**Results:** The types of medical services that family doctors should provide to women during pregnancy and in the postpartum period and the competencies that they should have to ensure the provision of these services were determined by experts. The most acceptable ways to acquire the necessary competencies, as well as the benefits and risks of providing these services at the primary level of health care were designated.

**Conclusions**: The implementation of the proposed types of medical care by family doctors will increase the availability and quality of medical care for women during pregnancy and in the postpartum period.

**KEY WORDS:** Women, pregnancy, postpartum period, medical care, family doctors, types of services, competencies

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## INTRODUCTION

In the course of reforming the health care system of Ukraine, the development of primary health care on the basis of general medical practice – family medicine has been identified as a priority [1,2]. At present, primary health care is legally and financially differentiated from specialized medical care and acquires a certain level of trust in the population of the country [3,4], which has the right to freely choose a family doctor [5].In this case, family doctor, being the doctor of the first contact of the population with the health care system, determines medical route of a patient [6]. At this stage of the reform, the Ministry of Health of Ukraine has approved the Procedure for providing medical care by family doctors [7], which includes the provision of primary obstetric and gynaecological medical care to women during pregnancy and in the postpartum period [8]. But so far the list of services in this area, which should be provided by a family doctor and competencies the specialist should have for this purpose, has not been determined.

The above mentioneddefined the relevance of this study.

## THE AIM

To determine expertly the types of medical services that family doctors should provide to women during pregnancy and in the postpartum period and the competencies they should have to provide these services. Identify the most acceptable ways to acquire the necessary competencies, as well as the benefits and risks of providing these services at the primary level of health care.

## MATERIALS AND METHODS

During the study, the method of expert assessment, statistical method and method of structural and logical analysis were used. Systematic approach became the methodological basis of the study. Using a specially designed questionnaire, the experts identified the types of medical services that family doctors should provide to women during pregnancy and in the postpartum period and the competencies they should have to ensure the provision of these services. As well as the most acceptable ways for family doctors to acquire the necessary competencies and the benefits and risks of providing these services at the primary level of care. 5 obstetricians-gynaecologists, 5 health care organizers, 5 family doctors and 3 scientists in the specialty "Social Medicine" took part in the development of the used questionnaire.Independent experts involved: 50 obstetricians and gynaecologists who provide women with outpatient care and 50 family doctors. Statistical processing of the results was performed using Microsoft Excel and Statistica – 6. Services and competencies, which are positively assessed by less than 50% of experts, are not recommended for use at the primary level of medical care.

**Table I.** Types of medical services to be provided to women during pregnancy and in the postpartum periodby family doctors (based on the results of expert assessment)

| Competences | Obstetricians - gynaecologists | | Family doctors | |
|---|---|---|---|---|
| | Absolute | %±m | Absolute | %±m |
| Informing women about the first signs of pregnancy | 47 | 94,0±1,2 | 49 | 98,0±0,7 |
| Motivation of women to seek medical attention early in the event of signs of pregnancy | 45 | 90,0±1,5 | 50 | 100,0 |
| Motivation of women to regular medical supervision during pregnancy | 45 | 90,0±1,5 | 50 | 100,0 |
| Introducing pregnant women to the optimal lifestyle during pregnancy | 43 | 86,0±1,7 | 46 | 92,0±1,4 |
| Providing advice on sexual activity during pregnancy | 37 | 74,0±2,2 | 41 | 82,0±1,9 |
| Recommendation for women on the optimal diet during pregnancy | 40 | 80,0±2,0 | 47 | 94,0±1,2 |
| Explaining to women the possible harm due to self-medication during pregnancy | 45 | 90,0±1,5 | 49 | 98,0±0,7 |
| In the presence of extragenital pathology, organization of consultations with relevant specialists | 47 | 94,0±1,2 | 50 | 100,0 |
| Organization of examination of pregnant women in accordance with the current Clinical Protocol | 43 | 86,0±1,7 | 45 | 90,0±1,5 |
| Informing married couples about the physiological course of pregnancy | 38 | 76,0±2,2 | 44 | 88,0±1,6 |
| Informing married couples about the physiological course of fetal development | 34 | 68,0±2,3 | 37 | 74,0±2,2 |
| Informing married couples about the symptoms of pregnancy complications | 47 | 94,0±1,2 | 49 | 98,0±0,7 |
| Informing married couples about the symptoms of fetal developmental disorders | 42 | 84,0±1,8 | 44 | 88,0±1,6 |
| Diagnosis of pregnancy | 44 | 88,0±1,6 | 47 | 94,0±1,2 |
| Diagnosis of fetal development | 38 | 76,0±2,1 | 41 | 82,0±1,9 |
| Referral of a pregnant woman for consultation with an obstetrician-gynaecologist in case suspecteddisorders of pregnancy or fetal development with determination of the method of transportation | 42 | 84,0±1,8 | 43 | 86,0±1,7 |
| Fulfilmentof the consulting doctor recommendations | 48 | 96,0±1,0 | 45 | 90,0±1,5 |
| Referral for routine hospitalization at high risk of complications or miscarriage | 40 | 80,0±2,0 | 46 | 92,0± |
| Referral to emergency hospitalization determining the method of transportation in the event of threatening symptoms during pregnancy | 48 | 96,0±1,0 | 50 | 100,0 |
| Home nursing of a pregnant woman in order to get acquainted with the living conditions of the pregnant woman and the conditions for the unborn child | 47 | 84,0±1,8 | 50 | 100,0 |
| In case of inappropriate social, material and sanitary and hygienic living conditions of a pregnant woman and unborn child - official notification of the social security authorities | 47 | 84,0±1,8 | 50 | 100,0 |
| Organization of medical supervision of women in the postpartum period | 41 | 82,0±1,9 | 44 | 88,0±1,6 |
| Providing recommendations on the optimal lifestyle and the course of the postpartum period | 41 | 82,0±1,9 | 44 | 88,0±1,6 |
| Diagnosis of postpartum complications | 40 | 80,0±2,0 | 43 | 86,0±1,6 |
| Informing women about the symptoms of postpartum complications | 40 | 80,0±2,0 | 44 | 88,0±1,6 |
| Determining tactics in the event of postpartum complications | 41 | 82,0±1,9 | 44 | 88,0±1,6 |
| Organization of the«School of Responsible Parenthood» | 47 | 94,0±1,2 | 49 | 98,0±0,7 |
| Cooperation with specialized obstetric (perinatal) institutions for the provision of medical care to women during pregnancy and in the postpartum period | 50 | 100,0 | 50 | 100,0 |
| Advanced training in providing medical care to women during pregnancy and in the postpartum period | 50 | 100,0 | 50 | 100,0 |

## RESULTS AND DISCUSSION

The first step in the study was establishing by independent experts a list of services provided by family doctors to women during pregnancy and in the postpartum period. The results obtained during the study are given in Table I.

The analysis of the data given in Table 1 indicates that the experts in obstetrics and gynaecology the highest evaluated the provision by family doctors of the following services to women during pregnancy and in the postpartum period: cooperation with institutions of specialized

**Table II.** Benefits of providing medical services to women during pregnancy and in the postpartum period by family doctors at the primary level of medical care (according to the results of expert assessment)

| Possible risks | Obstetricians - gynaecologists | | Family doctors | |
|---|---|---|---|---|
| | Absolute | %±m | Absolute | %±m |
| Increasing the territorial availability of medical care for women during pregnancy and in the postpartum period | 41 | 82,0±1,9 | 50 | 100,0 |
| Reduction of duplication of medical care for women during pregnancy and in the postpartum period | 35 | 70,0±2,3 | 39 | 78,0±2,1 |
| Improving resource efficiency | 27 | 54,0±2,5 | 35 | 70,0±2,3 |
| Reducing the level of untimely hospitalization of pregnant women with complications | 43 | 86,0±1,7 | 44 | 88,0±1,6 |
| Improving intra-sectoral cooperation in providing medical care to women during pregnancy and in the postpartum period | 37 | 74,0±2,2 | 45 | 90,0±1,5 |
| Higher level of early medical supervision of pregnant women | 44 | 88,0±1,6 | 46 | 92,0±1,4 |

**Table III.** Possible risks of providing medical services to women during pregnancy and in the postpartum period by family doctors at the primary level of medical care (based on the results of expert assessment)

| Possible risks | Obstetricians - gynaecologists | | Family doctors | |
|---|---|---|---|---|
| | Absolute | %±m | Absolute | %±m |
| Low level of interaction between primary care service and obstetrics and gynecology service | 44 | 88,0± 1,6 | 49 | 98,0±0,7 |
| Low level of professional readiness and qualification of family doctors | 47 | 84,0±1,8 | 33 | 66,0±2.4 |
| Low level of professional readiness and qualification of family nurses | 49 | 96,0±1,0 | 33 | 66,0±2,4 |
| Lack of motivation of primary health care workers to provide this area of medical services | 45 | 90,0±1,5 | 29 | 58,0±2,5 |
| Low level of public confidence in primary health care workers | 46 | 92,0±1,4 | 23 | 46,0±2,5 |
| Increased duplication of medical services for women during pregnancy and in the postpartum period | 42 | 84,0±1,8 | 29 | 58,0±2,5 |
| Low level of inter-sectoral medical cooperation in providing care to women during pregnancy and in the postpartum period | 45 | 90,0±1,5 | 42 | 84,0±1,8 |

obstetric (perinatal) care on providing medical assistance to women during pregnancy and in the postpartum period and advanced training in providing medical care to women during pregnancy and in the postpartum period (100.0%), referral to emergency hospitalization defining the method of transportation in case of threatening symptoms during pregnancy and fulfilment of consulting doctor's recommendations (96.0 ± 1.0%),in the presence of extragenital pathology, organization of consultations with relevant specialists, informing women about the first signs of pregnancy, informing married couples about the symptoms of pregnancy complications and organization of the "School of Responsible Parenthood" (94.0 ± 1.2%). Informing married couples about the physiological course of fetal development has received a low rating from experts of obstetrics and gynaecology (68.0 ± 2.3%), providingrecommendations on sexual activity during pregnancy at the primary level of medical care – (74.0 ± 2, 2%), informing married couples about the physiological course of pregnancy and diagnosis of fetal developmentby family doctors – (76.0 ± 2.2%).

The following services were fully supported by independent experts – family doctors for women during pregnancy and in the postpartum period at primary level of health care: motivating women to seek medical attention early in case of pregnancy, motivating women to see a doctor regularly during pregnancy, referral for emergency hospitalization with the definition of the method of transportation in the event of threatening symptoms during pregnancy,home nursing of a pregnant woman in order to get acquainted with the living conditions of a pregnant woman and the conditions for unborn child andofficial notification of social security authoritiesin case of inappropriate social, material and sanitary conditions of pregnant woman and unborn child (100.0%). Informing of married couples about the physiological course of fetal developmentby family doctors received the lowest level of support from both family doctorsand obstetricians and gynaecologists (74.0 ± 2.2%).

Next, the results of the expert assessment of the benefits of providing services to women during pregnancy and in the postpartum period by family doctors were analyzed. The results of the study are shown in Table II.

**Table IV.** Competences of family doctors to provide medical services to women during pregnancy and in the postpartum period (based on the results of expert assessment)

| Competences | Obstetricians - gynaecologists | | Family doctors | |
|---|---|---|---|---|
| | Absolute | %±m | Absolute | %±m |
| Ability to inform women about the first signs of pregnancy | 48 | 96,0±1,0 | 48 | 96,0±1,0 |
| Ability to motivate women to seek medical attention early in the event of signs of pregnancy | 46 | 92,0±1,4 | 49 | 98,0±0,7 |
| Ability to motivate women for regular medical supervision during pregnancy | 46 | 92,0±1,4 | 50 | 100,0 |
| Ability to familiarize pregnant women with the optimal lifestyle during pregnancy | 45 | 90,0±1,5 | 46 | 92,0±1,4 |
| Ability to provide advice to married couples on sexual activity during pregnancy | 35 | 70,0±2,3 | 42 | 84,0±1,8 |
| Ability to provide advice to women on the optimal diet during pregnancy | 40 | 80,0±2,0 | 47 | 94,0±1,2 |
| Ability to provide explanations to women of possible harm due to self-medication during pregnancy | 44 | 88,0±1,6 | 48 | 96,0±1,0 |
| Ability to detect the presence of extragenital pathology and organize consultations with relevant specialists | 48 | 96,0±1,0 | 50 | 100,0 |
| Knowledge of the current Clinical Protocol for the supervision of pregnant women with the physiological course of pregnancy | 45 | 90,0±1,5 | 45 | 90,0±1,5 |
| Ability to organize the examination of pregnant women in accordance with the current Clinical Protocol | 45 | 90,0±1,5 | 45 | 90,0±1,5 |
| Ability to inform married couples about the physiological course of pregnancy | 37 | 74,0±2,2 | 44 | 88,0±1,6 |
| Ability to inform married couples about the physiological course of fetal development | 35 | 70,0±2,3 | 39 | 78,0±2,1 |
| Ability to inform couples about the symptoms of pregnancy complications | 46 | 92,0±1,4 | 48 | 96,0±1,0 |
| Ability to inform couples about the symptoms of fetal developmental disorders | 42 | 84,0±1,8 | 44 | 88,0±1,6 |
| Ability to diagnose pregnancy | 46 | 92,0±1,4 | 45 | 90,0±1,5 |
| Ability to diagnose fetal development | 39 | 78,0±2,1 | 43 | 86,0±1,7 |
| Ability to refer a pregnant woman for consultation to an obstetrician-gynaecologist in case of suspected disorders of pregnancy or fetal development with determination of the method of transportation | 42 | 84,0±1,8 | 45 | 90,0±1,5 |
| Ability to ensure fulfilling the recommendations of consulting doctor | 50 | 100,0 | 45 | 90,0±1,5 |
| Ability to refer a woman to a planned hospitalization at high risk of complications or miscarriage | 41 | 82,0±1,9 | 45 | 90,0±1,5 |
| Ability to ensure the referral of a woman to emergency hospitalization with the definition of the method of transportation in the event of threatening symptoms during pregnancy | 48 | 96,0±1,0 | 50 | 100,0 |
| Ability to provide home nursing for pregnant women in order to get acquainted with the living conditions of the pregnant woman and the conditions for the unborn child | 48 | 96,0±1,0 | 50 | 100,0 |
| Ability to provide official notification of social security authorities in case of inappropriate social, material and sanitary-hygienic living conditions of a pregnant woman and conditions for unborn child | 48 | 96,0±1,0 | 50 | 100,0 |
| Ability to organize medical care for women in the postpartum period | 42 | 84,0±1,8 | 44 | 88,0±1,6 |
| Ability to provide advice to women on the optimal lifestyle and information about the postpartum period | 41 | 82,0±1,9 | 45 | 90,0±1,5 |
| Ability to diagnose postpartum complications | 42 | 84,0±1,8 | 43 | 86,0±1,7 |
| Ability to inform women about the symptoms of postpartum complications | 41 | 82,0±1,9 | 45 | 90,0±1,5 |
| Ability to determine the tactics of action in the event of postpartum complications | 42 | 84,0±1,8 | 44 | 88,0±1,6 |
| Willingness and ability to organize the work of the «School of Responsible Parenthood» | 48 | 96,0±1,0 | 49 | 98,0±0,7 |
| Ability to cooperate with institutions of specialized obstetric (perinatal) care in providing medical care to women during pregnancy and in the postpartum period | 50 | 100,0 | 50 | 100,0 |
| Willingness for advanced trainingon providing medical care to women during pregnancy and in the postpartum period | 50 | 100,0 | 50 | 100,0 |

**Table V.** The most acceptable ways to acquiring necessary competencies for the provision of medical services to women during pregnancy and in the postpartum period by family doctors (based on the results of expert assessment)

| Ways to acquire the necessary competencies | Obstetricians - gynaecologists | | Family doctors | |
|---|---|---|---|---|
| | Absolute | %±m | Absolute | %±m |
| Thematic improvement courses | 42 | 84,0±1,8 | 45 | 90,0± 1,5 |
| Short-term trainings on the basis of the regional training center | 45 | 90,0±1,5 | 43 | 86,0± 1,7 |
| Remote webinars | 30 | 60,0±2,4 | 36 | 72,0± 2,2 |
| Seminars in the primary care center | 26 | 52,0±2,5 | 45 | 90,0±1,5 |
| Scientific periodicals | 9 | 18,0±1,9 | 9 | 18,0± 1,9 |
| Methodical literature | 25 | 50,0±2,5 | 23 | 46,0±2,5 |
| Workplace internship in a women's clinic | 35 | 70,0±2,3 | 39 | 78,0±2,1 |
| Workplace internship in a maternity hospital | 19 | 38,0± 2,4 | 21 | 42,0±2,5 |
| Scientific and practical conferences | 11 | 22,0±2,1 | 8 | 16,0±1,8 |
| Teleconsultations with obstetricians and gynecologists | 25 | 50,0±2,5 | 32 | 62,0±2,4 |

Analysis of the results of a survey of independent experts showed that most experts as an advantage noted the increased territorial availability of medical care for women during pregnancy and in the postpartum period, while obstetricians and gynaecologists praised the following benefits: higher level of early medical supervision of pregnant women ( 88.0 ± 1.6%) and a decrease in the level of untimely hospitalization of pregnant women with complications (86.0 ± 1.7%), and family doctors pointed at such advantages as a higher level of early medical supervision of pregnant women (92.0 ± 1.4%) and improvement of intra-sectoral cooperation in providing medical care to women during pregnancy and in the postpartum period (90.0 ± 1.5%).

According to set aim, the next step of the study was to identify possible risks of providing medical services to women during pregnancy and in the postpartum period by family doctors. The obtained data are given in table III.

Experts obstetricians and gynaecologists rated the highest risks as low level of professional readiness and qualification of family nurses (96.0 ± 1.0%), low level of public confidence in primary care (92.0 ± 1.4%), lack of motivation of primary care employees to provide this area of medical services and low level of inter-sectoral medical cooperation in providing care to women during pregnancy and in the postpartum period (90.0 ± 1.5%). Family doctors indicated possible risks such as low level of interaction between primary care and obstetrics and gynaecology service (98.0 ± 0.7%), low level of inter-sectoral medical cooperation in providing care to women during pregnancy and in the postpartum period (84.0 ± 1.8%), low level of professional readiness and qualification of family doctors and family nurses (66.0 ± 2.4%).

Based on the data obtained during the study, the competencies required by family doctors to provide medical services to women during pregnancy and in the postpartum period were identified. The results of the experts' assessment of the competencies required by family doctors are given in Table IV.

The analysis of the competencies recommended by experts – family doctors and their comparison with the list of medical services for women during pregnancy and in the postpartum period indicated their certain compliance. This means that the experts recommended acquiring by family doctors of competencies necessary to provide the medical services recommended by them.

We also examined the most acceptable ways for family doctors to acquire the necessary competencies to provide medical services to women during pregnancy and in the postpartum period. The results obtained are given in Table V.

According to the experts both obstetricians and family doctors, the most acceptable ways for family doctors to acquire the necessary competencies to provide medical services to women during pregnancy and in the postpartum period are thematic improvement courses, short-term trainings on the basis of the regional training centre, remote webinars and internships for family doctors in the workplace in a women's clinic. Experts rated the possibility of using scientific-practical conferences and scientific periodicals to acquire the necessary competencies the lowest.

## CONCLUSIONS

The study identified the types of medical services that family doctors should provide to women during pregnancy and in the postpartum period and the competencies they should have to provide these services and the most acceptable ways to acquire the necessary competencies, as well as the benefits and risks of providing these services at the primary level of medical care. The implementation of the proposed types of medical care by family doctors will increase the availability and quality of medical care for women during pregnancy and in the postpartum period.

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ORIGINAL ARTICLE

# THE EFFECT OF ATORVASTATINUM IN THE TREATMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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## ABSTRACT

**The aim:** Was to evaluate the effect of 6-month pathogenetic treatment in combination with atorvastatinum on the endothelium function, lipid and adipokine levels, paroxonase activity and activity of inflammatory process in RA patients.

**Materials and methods:** The study included 55 patients with RA, dividing into two groups depending on the intended therapy. The first group included 33 patients with "traditional" treatment by methotrexate, glucocorticoids, and non-steroid anti-inflammatory drugs. The second group included 22 patients with "traditional" treatment and additionally prescribed of atorvastatinum 20 mg/day. The lipid profile, leptin, adipokine, paroxonase activity. C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) levels, FMDBA and IMT of carotid artery were determined in all participants of the study. Control parameters were recorded before the start, after 1 and 6 months of treatment.

**Results:** The FMDBA has increased by 32% in the second group, compared by only 10.9% in the first group. The dynamics of IMT in the first group was also twice lower than in group with the additional use of atorvastatinum. The leptin levels in the second group significantly decreased by 27% and adiponectin levels increased by 12.8%, than in the first group – by 12.8% and by 7% respectively. The appointment of statins over 6 months resulted in DAS28, TNF-α, ESR and CRP reduction by 15%, 31%, 25% and 21.5% respectively. In the first group the dynamics of indicate rates ranged from 7.8% to 22.5%, and was significantly lower than in the second group.

**Conclusions:** As a result of the study, it was found that the appointment of atorvastatinum 20 mg/day during 6 months not only reduces dyslipidemia, but also significantly reduces the inflammatory process and *adipokine dysregulation*, normalizes serum paraoxonase activity and improves the endothelium function.

**KEY WORDS:** metabolic desorders, inflammatory, endothelium dysfunction, statins

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## INTRODUCTION

Cardiovascular events are known to be the main cause of mortality in patients with rheumatoid arthritis (RA) [1, 2, 3]. The main causes of the accelerated atherosclerotic process in RA patients are the result of the interaction between traditional risk factors of cardiovascular disease (dyslipidemia, arterial hypertension, limited physical activity, obesity) and chronic inflammation due to RA [4, 5, 6]. The prominent role of chronic inflammation in the pathogenesis of accelerated atherosclerosis in RA patients emphasizes the necessity of a modified approach in the management of patients, espacially to control not only traditional cardiovascular risk factors, but also the impact on a powerful autoimmune inflammatory process. There were studies which have justified the appropriateness of lipid metabolism disorders correction in RA patients with statins, which are known not only inhibit the synthesis of cholesterol, but also able to block the inflammatory process in the vascular wall and prevent the transformation of cellular elements in vessels [7, 8].

Data on the possible statin influence on adipokine level, serum paraoxonase activity was not detected, and the role of this group of drugs in reducing of the atherosclerotic progress in RA patients is discursive [9].

## THE AIM

In view of this, the aim of the study was to evaluate the influence of 6-month pathogenetic treatment in combination with atorvastatinum on the endothelium function, lipid metabolism, adipokine levels, serum paroxonase activity, and inflammatory process in patients with RA.

## MATERIALS AND METHODS

An "open"-controlled study was conducted, which included 55 patients with RA. The seropositive type of RA was diagnosed in 47 (85.5%) patients. All patients were divided into two groups depending on the intended therapy. The first group included 33 patients who received «traditional» treatment in accordance with the clinical protocol "Rheumatoid arthritis" (Order of the Ministry of Health of Ukraine dated April 11, 2014, No. 263), which included methotrexate 10-15 mg/week; glucocorticoids (GC) at a stable dose not exceed 10 mg/day by prednisolone; non-steroid anti-inflammatory drugs (NSAD) in a stable dose. The second group consisted of 22 patients, who with «traditional» treatment was additionally prescribed of atorvastatinum in dose 20 mg/day. The dose of GC, methotrexate and NSAD was stabile during the study

period. The duration of the controlled study was 6 months. The control rates were recorded before, after 1 and 6 months of treatment. The adiponectin level was determined in blood serum by immunoassay using AssayMax Human Adiponectin (ASSAYPRO, USA); leptin level was determined using standard sets of the "DRG", Germany. Serum C-reactive protein levels (CRP) and tumor necrosis factor-alpha (TNF-α) were detected by immunoassay using standard sets.

The total cholesterol level (TC), high density lipoprotein cholesterol (HDLP), and triglycerides (TG) in serum were determined according to the standard methodology. The value of low density lipoprotein (LDLP) was calculated according to the Friedwald formula: LDLP=TC-HDLP-0.45*TG. The activity of paraoxonase-arilesterase (CF 3.1.1.2) in serum was determined by spectrophotometric method [10].

High resolution ultrasound and Doppler ultrasonography of the brachial artery by D. Celermajer [11] were performed to study endothelium function. Flow-mediated vasodilation of the brachial artery (FMDBA) was assessed according to changes in its diameter and measured before and after temporary occlusion of the vessel with blood preasure cuff (reactive hyperemia). Location of the brachial artery was associated with visualization of its internal diameter and was measured in the middle third of the shoulder. Sonographic B-mode scanning and pulsed Doppler ultrasound of blood flow spectra were done on ultrasound scanner "Sonoline 6000 C" (Medisson, Southern Korea) on the 30th, 60th and 90th seconds after cuff decompression. Brachial artery dilation by more than 8% from baseline diameter in 30 seconds after decompression was considered to be the criteria of adequate endothelial response to ischemia. All measurements of endothelial relaxation were done from 8 to 10 a.m.

The thickness of intima-media complex (IMT) of the common carotid artery was determined at the time of B-mode ultrasonography of carotid artery in diastole 2sm from bifurcation at maximum magnification. The area of atherosclerotic plaques of carotid artery (cAP) was measured in all the patients, and the extent of vascular atherosclerotic damage was evaluated [12]. Echocardiography (EchoCG) was done for 63 patients with RA on ultrasound scanner "Sonoline 6000 C" (Medisson, Southern Korea) Statistical processing of the obtained results was carried out on a personal computer using the standard statistical programs. The results are presented as the mean ± standard error (m ± SE). All values were follow a normal distribution. The average value, standard errors, reliability of the differences were evaluated according to Student's t-criterion. Pearson's correlation coefficient test was used to measure the strength of a linear association between two variables. The statistical significance was determined if $P<0,05$

## RESULTS

As can be seen from the data in Table I, the study groups were representative by age, gender, major severity, disease activity and laboratory data at the start of treatment. At the first stage of the study, we evaluated the influence of individual treatment on the dynamics of lipid metabolism. (Table II). Prior to the treatment, the levels of TC, LDLP, HDLP and TG were approximately the same in all study groups.

The «traditional» therapy had the slightest effect on the dynamics of lipid metabolism rates. After 6 months of treatment, the level of TC, LDLP and TG decreased by 4.2% -6.5% on average, while HDLP increased by 4.3%.

The inclusion of atorvastatinum in the complex of treatment after 1 month provided a significant decrease in the level of TC (by 5.6%), LDLP and TG (by 8.2%) and an increase in HDLP levels (by 10.9% ), and after 6 months – by 16.8-21.6% respectively, which was significantly higher than in group with «traditional» treatment.

The influence of different treatment options on the dynamics of adipokin levels and serum paroxonase activity was also evaluated (Table III). It was established that «traditional» treatment had little effect on the dynamics of leptin levels, adiponectin levels and paraoxonase activity. After 6 months of pharmacotherapy, adiponectin levels increased by 7%, leptin levels decreased by 12.8%. The activity of serum paroxonase also increased by 7% after 6 months of treatment.

At the same time, the additional treatment with atorvastatinum significantly influenced the dynamics of adipocyne levels and paraoxonase activity. The leptin level significantly decreased by 27% and adiponectin leptin increased by 12.8% after 6 months of atorvastatinum use. Regarding the activity of paraoxonase, it has probably increased by 21% after 6 months of treatment.

The influence of different treatment options on the parameters of endothelial function (FDVBA, IMT of carotid artery) and the area of atherosclerotic plaques (AP) in the common carotid artery was evaluated (Table IV). The data from the tables 4 show that within 6 months, FMDBA has increased in both groups, regardless of pharmacotherapy. The largest, and significantly better, dynamics of the analyzed parameters was seen in the group of patients treated with atorvastatinum. In particular, the level of FMDBA in response to the test of reactive hyperemia has increased by 32%, while in the group of patients receiving «traditional» therapy only by 10.9%. The dynamics of IMT in the last group was also twice lower than in the group with the additional use of atorvastatinum. Less dynamic under the influence of treatment were in the area of AP. Traditional treatment was accompanied by the lower dynamics of the analyzed criterion, and the appointment of atorvastatinum for 6 months probably stabilized the size of AP.

Subsequently, the influence of different treatment options on the dynamics of the inflammatory process (ESR, CRP, TNF-α, DAS28) was evaluated (Table V). It was established that positive dynamics of clinical and laboratory parameters of inflammatory activity took place in both studied patients groups. It was lower in the group with traditional treatment and more prominent in the group of patients receiving atorvastatinum additionally. The appointment of statins over 6 months resulted in DAS28, TNF-α, ESR and CRP reduction by a 15%, 31%, 25% and 21.5% respectively.

In the group of patients receiving only traditional therapy, the dynamics of above indicated rates ranged from 7.8% to 22.5%, and was significantly lower than in the group with the additional appointment of atorvastatinum.

## DISCUSSION

Thus, during the study, it was found that the effectiveness of the pharmacotherapy varied significantly depending on the treatment. Traditional therapy in patients with RA significantly reduced the intensity of the inflammatory response and to a lesser extent influenced the dynamics of the lipid spectrum, the levels of adipokines, the activity of serum paroxonase, and the functional capacity of the endothelium.

The inclusion of atorvastatinum in a complex treatment allows to achieve the target lipid levels in most patients with RA. We found that treatment with atorvastatinum over 6 months of treatment reduced the levels of TC (by 19.5%), LDLP (by 23.4%), TG (by 17.4%), and HDLP increased by 16.8% ), which was in 3-4.1 times higher than in the group of patients receiving pathogenetic treatment without atorvastatinum. According to all indicators, the differences between the groups were reliable. In general, the data we have received coincide with the data of other researchers. In particular, the administration of statins reduced TC and LDLP levels by 27-38% in patients with RA [13, 14, 7]. The 6-week use of atorvastatinum in patients receiving *Janus kinase inhibitors* reduced LDLP by 35.2%, while in the group, with no additional use of atorvastatinum, it was only 5.8% [15]. According to Akiyama M., et al. (2015), the use of atorvastatinum with the traditional treatment, including immunobiologic agents also significantly influenced the dynamics of all lipid metabolism parameters in RA patients [7].

According to our data, the use of atorvastatinum significantly improved the functional capacity of the endothelium, with little effect on the IMT of carotid artery and the area of atherosclerotic plaques. After 6 months of treatment, FMVDBA increased by 32.4%. In our opinion, the relatively low dynamics of the IMT and the AP area is obviously related to the short period of statin using in our study. At the same time, according to the literary data, the appointment of hypolipidemic therapy in RA patients and ankylosing spondylitis during 18 months not only allowed to reach the target levels in most patients, but also provide regression of atherosclerotic plaques [16]. It is also reported that long-term administration of statins can prevent the development of endothelial dysfunction [13], as well as reduce cell adhesion-1 and fibrinogen molecules [17]. Although there are some studies that do not confirm the improvement of subclinical atherosclerotic vascular damage in response to the appointment of statins [9].

We have shown the ability of atorvastatinum to reduce the leptin concentration and increase adiponectin in blood serum of patients with RA. In particular, leptin levels in the second group significantly decreased by 27%, compared to 12.8% in the first group; adiponectin concentration increased by 12.8% in the second group, but it was only 7% in the first group. The positive role of statins in restoring adipokin status is also demonstrated in experimental studies [18]. Statins act directly on white adipocytes in humans, regulate adipokine secretion and reduce the expression of leptin [19], although there are such studies , which deny the relationship between statin administration and leptin secretion [20]. According to literature data the using of atorvastatinum has contributed to the leptin normalization in patients with RA [14].

One of the important pleiotropic statin effects that prevent the development of cardiovascular complications is increasing of serum paraoxonase activity [21]. According to Park and co-authored data, (2016) the using of rosuvastatin at a dose 10 mg / day for 8 weeks increased paraoxonase activity by 19.1% [22]. We also found the ability of atorvastatinum to increase the activity of paraoxonase in serum. In particular, after 6 months of treatment, the paraoxonase activity increased by 7% in the first group, whereas in the second group by 21%.

The hypolipidemic therapy significantly accelerated the dynamics of clinical manifestations of the disease (DAS28) and laboratory parameters of the inflammatory process. Thus, under the influence of atorvastatinum, the serum TNF-α concentration decreased by an average of 30%, CRP – by 21%, ESR – by 25%, while, the dynamics of disease activity laboratory parameters was less clear during the traditional therapy, and for the TNF -alpha, CRP and ESR it ranged from 15.8-22%. Other researchers point out the ability of statins to suppress the inflammatory marker synthesis in patients with RA [14]. In particular, according to McCarey et al. (2004) in the group of patients receiving atorvastatinum 40 mg/day the total inflammatory activity index DAS28 decreased by 31% and the CRP level decreased by 50% while in the group of patients without statins DAS28 decreased by only 10%, and CRP by 28% [23].

Thus, our data has shown that traditional therapy with the use of basic agents, GC and NSAIDs had a fairly moderate influence on the lipid spectrum, adipokine level, paraoxonase activity and endothelial dysfunction in patients with RA. Inclusion of atorvastatinum 20 mg/day during 6 months has resulted in decreasing of dislipoproteinemia, the severity of *adipokine dysregulation* and inflammation, normalizing serum paroxonase activity and reducing of atherosclerotic damage.

## CONCLUSIONS

1. The pathogenetic therapy with methotrexate, GC, nonsteroidal anti-inflammatory drugs has significantly reduced the inflammatory response in RA patients (DAS28, ESR, CRP, TNF-α) but less influenced the dynamics of lipid and adipokine spectrum, serum paroxonase activity and functional endothelium status.
2. The administration of atorvastatinum in dose 20 mg/day during 6 months of treatment has not only reduced the TC (19.5%), LDLP (23.4%), TG (17.4%), and in-

creased the HDLP (by 16,8%), but also significantly reduced the inflammatory process (DAS28, ESR, CRP, TNF-α), removed *adipokine dysregulation*, normalized serum paroxonase activity, and improved endothelium functional status

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ORIGINAL ARTICLE

# ASSOCIATION BETWEEN SINGLE POLYMORPHISM IN THE LOCUS RS17216473 OF THE GENE THAT ENCODES 5-LIPOXYGENASE-ACTIVATING PROTEIN AND RISK OF MYOCARDIAL INFARCTION

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## ABSTRACT

**The aim:** To study the association between A/A, G/A, A/A genotypes, alleles A, G of the SNP rs17216473 of the gene that encodes ALOX5AP and the risk of myocardial infarction within the Ukrainian population.

**Materials and methods:** PCR in real time and the analysis to discriminate alleles were used. The statistical processing was carried out by χ2 criteria and by χ2 criteria with Yates correction.

**Results:** For the first time the SNP rs17216473 of gene that encodes ALOX5AP has been established to be statistically significantly associated with the risk of myocardial infarction in Ukrainian population. The connection with genotype A/A was opposite to that with genotype G/G. That is, A/A contribution to myocardium infarction has been statistically significant whereas, G/G has been statistically significantly associated with the absence of myocardial infarction. G/A genotype has not been statistically significantly associated with myocardial infarction. It has also been established a statistically significant connection exists between the risk of myocardial infarction and the presence of allele A (minor allele) of the polymorphism. Allele G, however, has a statistically significant association with the absence of myocardial infarction. All humans-homozygotes with the minor allele A had suffered from myocardial infarction. In the control group, humans-homozygotes with the minor allele A were not found.

**Conclusions:** Summarizing our obtained results, we assume the carriers of G/G genotype to have a minimal risk of myocardial infarction onset, the carriers of G/A genotype to have a moderate risk and the carriers of A/A to have a great risk.

**KEY WORDS:** single nucleotide polymorphism, getetics, myocardial infarction

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## INTRODUCTION

The incidence of cardiovascular diseases is increasing, and the causes are varied [1, 2].

Single nucleotide polymorphisms (SNP) are directly related to the development of cardiovascular diseases. The SNP of the ALOX5 and ALOX5 AP genes that encode 5-lipoxygenase and its activating protein are assumed also to contribute to these diseases onset [3, 4, 5].

The areas of polymorphic loci (SNP) of the ALOX5AP and ALOX5 genes are clearly specified, and the quantity of such loci is extremely high.

One of the research methods is to establish an association between different polymorphic locuses of the corresponding genes and the diseases of the cardiovascular system. Herewith, one analyzes the statistical relationship between the corresponding SNP and myocardial infarction, stroke, atherosclerosis, etc.

Helgadottir A and co-authores were the first to show SNP ALOX5AP gene to be connected with the risk of myocardial infarction [3]. This association has been confirmed [4, 6], but some authors [5, 7, 8] deny the connection between SNP ALOX5AP and myocardial infarction. It has been stated that the correlation between SNP ALOX5AP and C-reactive protein exists [9]. Data exists regarding SNP ALOX5AP gene`s contribution to coronary artery disease [10, 11, 12], including the threat of its onset [13], there is also data stating an absence of such a contribution, but the presence of the connection between SNP ALOX5 gene and coronary artery disease [14]. Despite this, according to the literature data, some polymorphisms of the ALOX-5AP and ALOX5 genes are reported to be associated with cardiovascular diseases, including myocardial infarction.

The exact contribution of rs17216473 of ALOX5AP gene to the cardiovascular diseases development was mentioned in the following works. Tsai and coauthors [11] who had examined European-Americans pointed that SG13S377 specifically has been significantly linked to the risk of ischemic heart disease. This correlation was specified to be independent from other polymorphisms. Conversely, Huang with co-authors [6], who had examined the Chinese, showed the absence of the connection between rs17216473 and ischemic heart disease. The absence of any statistically significant association between SNP rs17216473 ALOX-5AP gene and myocardium infarction in a polyethnic

group was reported by David R. Crosslin and co-authores [12]. Concurrently, myocardial infarction, specifically in Europeans was verified to depend highly on haplotype B (rs17216473 (A), rs10507391 (A), rs9315050 (A) and rs17222842 (G)). However, the other polymorphism of haplotype B was specified to be the most significant [12].

The majority of the data referres to haplotypes and there are few studies done that would allow one to clearly link genotypes AA, AG or GG of rs17216473 polymorphism to cardiovascular diseases, or, vice versa, would show the absence of such an association. In our opinion, the study of particular SNP genotypes and alleles is just as important as the study of haplotypes.

The polymorphic nucleotide is in intron, therefore rs 17216473 is an intron polymorphism.

There are three genotypes in accordance with polymorphic nucleotide: AA, AG, GG and two alleles – A and G. The rest of this sequence is identical in all members of the Homo sapiens species.

The relationship between these genotypes and the risk of developing cardiovascular diseases is of great significance, since it is precisely such an association that will accurately answer the question; is this polymorphism related to the development of these diseases, or are they caused by other polymorphisms of ALOX5AP gene?

Polymorphism of this particular locus of chromosome 13, where the rs17216473 of the ALOX5AP gene is located, has been insufficiently studied. There are only few works where this polymorphism is mentioned.

There is a total absence of any data regarding the contribution of SNP rs 17216473 (SG13S377) of the ALOX5AP gene to the development of cardiovascular diseases in the Ukrainian population.

The study of the connection between rs17216473 polymorphism (SG13S377) of the ALOX5AP gene and the risk of cardiovascular diseases development in the Ukrainian population has been carried out for the first time only very recently.

In the present work, we aimed to study the association between the single-nucleotide polymorphism rs17216473 of the 5-lipoxygenase activating protein (ALOX5AP) gene and the risk of myocardial infarction among representatives of the Ukrainian population. To this end, we checked the presence or absence of a statistically significant association between the single nucleotide polymorphism (A/G) rs17216473 (SG13S377) of the ALOX5AP gene and the risk of myocardial infarction within the Ukrainian population.

## MATERIALS AND METHODS

### CONTINGENT UNDER THE STUDY: MYOCARDIAL INFARCTION GROUP, CONTROL GROUP

Leukocytes of human whole blood were researched in 95 people with myocardial infarction (myocardial infarction group) and in 110 individuals of the control group. Blood collection was carried out by medical staff at medical institutions.

Blood sampling was carried out in patients with myocardium infarction who were being treated in the hospital of National Scientific Center «M.D. Strazhesko Institute of Cardiology», department of cardioreanimation, the department of therapy № 4 of Bogomolets National Medical University. Blood sampling for the control group was carried out in healthy donors of corresponding age in the Kyiv City Center of Blood.

### REAGENTS

The following reagents were used in the work: the kit DIAtom DNA Prep («Isogene», Russia) having contained lysing reagent (Guanidine isothiocyanate), sorbent (*NucleoS*), saline solution to wash off DNA, ExtraGene to detach DNA from the sorbent, the mixture of probes for PCR TaqMan® for PCR. To prepare water solutions one used deionisation water.

### GENOTYPING

DNA was extracted from blood samples using the kits DIAtom DNA Prep («Isogene», Russia). The kit contained lysing reagent guanidine isothiocyanate, sorbent *NucleoS*, saline solution and ExtraGene to detach DNA from the sorbent. The method is based on the usage of guanidine isothiocyanate as a lysing reagent for cell lysis, solubilization of cell debris, and denaturation of cell nucleases. DNA is actively being absorbed on *NucleoS*™ under the presence of lysing reagent and then easily washed off from proteins with saline solution with ethanol. Subsequently, the DNA was extracted from the sorbent and transferred to sterile DNA-free and RNA-free microtest tubules («Eppendorf», USA). The obtained DNA was used for PCR in real time.

One determined the allele polymorphism of ALOX5AP gene (G→A) having used probes TaqMan® SNP Assay C_11599359_10. Amplification was performed with 7500 Fast Real-time PCR System («Aplied Biosystems» USA).

To clarify which of nucleotides, A or G, was present in the locus rs17216473 of ALOX5AP gene we determined the genotypes in the researched groups (the myocardial infarction group and the control group). For this purpose one made analysis to discriminate alleles with 7500 Fast Real-time PCR System Software (Applied Biosystems Foster City, USA) and counted the amount of the alleles and the quantity of people having had the corresponding genotype (A/A; A/G; G/G) within the healthy group (the control) and the patients with myocardium infarction. The contribution of three said genotypes and A and G alleles is being analyzed in the current study.

### STATISTICAL ANALYSIS

In order to establish the association between the genotypes G/G, A/G, A/A, alleles A and G in the polymorphic locus of rs17216473 of ALOX5AP gene and the risk of myocardial infarction we compared the control groups with myocardial infarction groups separately for each genotype by $\chi 2$ criteria

**Table I.** The distribution of genotypes G/G, G/A and A/A in the control group and in the miocardial infarction group

| The genotype | G/G | | G/A | | A/A | |
|---|---|---|---|---|---|---|
| The researched groups (N= the researched individuals) | The number of genotype carries, individuals | The percentage (%) of genotype carries in the group | The number of genotype carries, individuals | The percentage (%) of genotype carries in the group | The number of genotype carries, individuals | The percentage (%) of genotype carries in the group |
| The control groups N=110 (total amount) | 89 | 80,9 | 21 | 19,1 | 0 | 0 |
| Myocardial infarction groups N=95 (total amount) | 63 | 66,3 | 24 | 25,3 | 8 | 8,4 |
| Total amount of control and myocardial infarction groups N=205 | 152 | 74 | 45 | 22 | 8 | 4 |

**Table II.** The distribution of G and A alleles in the control group and in the myocardial infarction group

| Alleles | Allele G | | Allele A | |
|---|---|---|---|---|
| The researched groups (N= the amount of alleles) | The amount of G alleles in the group | The allele G percentage in the group (%) | The amount of A alleles in the group | The allele A percentage in the group (%) |
| The control group N=220 (the total amount) | 199 | 90,5 | 21 | 9,5 |
| The myocardial infarction group N=190 (the total amount) | 150* | 79 | 40* | 21 |

*- statistically significant difference between A and G alleles , $p<0,005$.

and by $\chi 2$ criteria with Yates correction. For this purpose, we built the four-fold contingency tables were in a such separate group we compared the genotype from the control group (health), genotype of myocardial infarction group, the carriers of other genotypes (not having this genotype) from the control group and myocardial infarction group respectively. In order to state the association between the alleles and myocardial infarction we compared the amount of A and G alleles in the control group and myocardial infarction group respectively. The statistical significance was estimated by $\chi 2$ criteria (Pearson criteria). The association was considered statistically significant under $p<0.05$.

## RESULTS

The number of genotype G/G carriers in the control group made 89 individuals or 80.9% of all carriers of G/G, G/A, A/A genotypes that was 110 individuals. There were also 21 G/A carriers in the control group or 19.1 % of all genotypes carriers. The carriers of A/A genotype were not found among healthy individuals. In myocardial infarction group the genotype A/A was determined in eight individuals, which made 8.4% of all carriers of researched genotypes (the total amount is 95 individuals), the number of G/G genotype carriers made 63 individuals or 66.3%, there were also 24 carriers of G/A genotype that is 25.3% respectively (Table I).

In the control group, the amount of the alleles G was 199 which made 90.5 % of the total amount (220 alleles) in this group (G and A). The amount of the alleles A in the control group made 21 and 9.5 % respectively of the all the alleles in the group.

In the myocardial infarction group, the amount of the G alleles was statistically significantly less than in the control and made 150. The percentage of G alleles was also lower in comparison with the control and made 79 % of all the alleles in this group (the total amount is 190). On the contrary, the number of alleles A in myocardial infarction group was statistically significantly higher than in the control group and made 40 or 21% of all the alleles in this group respectively (Table II).

At the current stage of the study we wanted to know whether the myocardial infarction is due to or whether there is any connection between the risk of myocardial infarction and the genotype or/and the alleles of the examined patient. That is, whether nucleotide G substitution for A in the rs17216473 locus of the ALOX5AP gene can contribute to the myocardial infarction.

For this purpose, we studied the absence or presents of the statistically significant association between the genotypes G/G, A/G, A/A and the alleles A and G in polymorphic locus rs17216473 of ALOX5AP gene and myocardial infarction.

**Table III.** The association between G/G, G/A, A/A genotypes and myocardial infarction by Pearson criteria (χ2)

| The genotype | With myocardial infarction (the number of individuals) | Without myocardial infarction (the number of individuals) | χ2 (The value of criteria/ p) | χ2 with Yates correction. (The value of criteria/ p ) |
|---|---|---|---|---|
| G/G | 63 | 89 | 5.663/0.018 (p<0,05)* | 4.927/0.027 (p<0,05)* |
| Not G/G | 32 | 21 | | |
| G/A | 24 | 21 | 1.134/0.288 (p>0.05) | 0.802/0.371 (p>0.05) |
| Not G/A | 71 | 89 | | |
| A/A | 8 | 0 | 9.639/ 0.002 (p<0.005)** | 7.525/0.007 (p<0.05) |
| Not A/A | 87 | 110 | | |

*- statistically significant difference between G/G genotype and other ones (not G/G) , p<0,05.
**- statistically significant difference between A/A genotype and other ones (not A/A), p<0,05.

**Table IV.** The association between A and G alleles and myocardial infarction by Pearson criteria (χ2)

| The allele | With myocardial infarction (the amount of alleles) | Without myocardial infarction (the amount of alleles) | χ2 (The value of criteria/ The level of significance) | χ2 with Yates correction. (The value of criteria/ The level of significance) |
|---|---|---|---|---|
| A | 40 | 21 | 10.660/0.002 (p<0.005)* | 9.770/ 0.002 (p<0.005)* |
| G | 150 | 199 | | |

*- statistically significant difference between A and G alleles , p<0,005.

The analyzing of the connection between G/G, G/A, A/A genotypes and myocardial infarction showed the presence of statistically significant association between A/A genotype and the myocardial infarction and, on the contrary, G/G genotype was established statistically significantly rarely in individuals with myocardial infarction than the other genotypes (A/G and A/A) (Table III).

As it is seen, two monozygous genotypes were shown to link to myocardial infarction wherein the connection with A/A was opposite to that with G/G. That is, A/A contribution to myocardium infarction was statistically significant and, on the contrary, G/G was statistically significantly associated with the absence of myocardial infarction (Table III).

Taking into consideration said above we checked the association between A and G alleles, and myocardium infarction. The statistically significant connection between A and G alleles and myocardium infarction was established, and as for the genotypes, the A allele contribution to myocardial infarction was opposite to that with G, namely, A was statistically significantly associated with myocardium infarction and G, vice-a-versa, was statistically significantly linked to the absence of it (Table IV).

## DISCUSSION

Genotype G/G/ was the most widely distributed among our researched individuals. The total number of carriers of this genotype (from the control and myocardial infarction groups) was 152, 74 % of the total number of all researched persons (205). Genotype G/G also prevailed in the control (healthy), totaling 80.9 %. Its domination among the healthy people was larger than that in the total group (80.9% and 74 % respectively). Genotype G/G was also present in myocardial infarction group (63 individuals) at 66.3 % (Table I), but its percentage became lower in myocardium infarction group compared to the healthy group and total group. Therefore, it is necessary to clarify, whether G/G genotype contributes to the prevention of myocardial infarction or whether it is a good prognostic sign to avoid myocardial infarction. There is a statistically significant difference between G/G and other genotypes for myocardial infarction (Table III), namely, the absence of myocardial infarction. Thus, G/G genotype can be considered dependable prognostic marker that essentially decreases the risk of myocardial infarction onset (Table III).

When analyzing the myocardial infarction groups, one sees that G/G was also prevalent (66.3% against 25.3% for G/A, 8.4% for A/A respectively) (Table I). This fact can be explained by, first, this genotype`s great distribution in Ukrainian population, and second, that the SNP polymorphism of rs17216473 of ALOX5AP gene is not the only that contributes to myocardium infarction and other cardiovascular diseases onset. The other polymorphism (rs1722842) of haplotype B was shown to be sufficiently associated with ishemic heart disease [6]. David R. Crosslin and co-authores [12] showed a very high dependence between haplotype B and myocardial infarction in Europeans, but the most significant was the other polymorphism [3, 4] established the association between an increased risk of myocardial infarction in Icelandic population and haplotype A, which contains rs17222814, rs10507391, rs4769874 and rs9551963 polymorphisms. According to the data that was received by meta-analysis of Chinese population by Huang and co-authors [6] the rs17222814G, rs10507391T, rs4769874G, rs9551963A polymorphisms of haplotype A were linked to myocardial infarction. Thus, the carriers of the G/G genotype from the myocardial infarction group can also be carriers of other SNP of ALOX5P gene.

G/A genotype was not as widely distributed as G/G, having only consisted of 45 individuals and 22 % of all the researched. The genotype presence increased in myocardial infarction group (24 individuals and 25.3 % of all the researched) and decreased in the control one (21 individual and 19.1 % of all the researched) (Table I). However, its contribution to myocardial infarction onset is not essential; there was no statistically significant difference between G/A genotype and other genotypes (Table III).

A/A genotype`s distribution was significantly less than others, having made only eight individuals or 4 % of total researched individuals. All of the A/A carriers were in the myocardial infarction group (8.4 % of all events of myocardial infarction), and there were none of them in the control group (Table I). The association between A/A genotype and myocardial infarction showed a statistically significant difference between A/A genotype and other genotypes (Table III). Interestingly, all A/A caries were only found in the myocardial infarction group. This can be explained, on the one hand, by A/A genotype`s rare distribution and, on the other hand, by their absence among donors of blood. There were carriers of the other two genotypes among donors that is naturally occurring. Of course, we can`t make the conclusion that there are no healthy people of the corresponding age among the carriers of A/A genotype, but at the same time, A/A genotype can be a serious prognostic sign for the onset of myocardial infarction or/and other cardiovascular diseases. We believe that the association between A/A genotype and cardiovascular diseases is worthy of being studied further. We cannot exclude the possibility that this genotype by itself causes the onset of some cardiovascular diseases, in particular, myocardial infarction upon reaching a certain age.

Thus, two monozygous genotypes were found to play the opposite role in an onset of myocardium infarction, that is, G/G counteracts to this disease development and A/A controversially, contributes to it.

Therefore, a minor allele was assumed to play a key role in myocardial infarction onset and G allele was believed presumably to be as defensive factor. A allele was found almost twice as often in patients with myocardial infarction (40 cases) compared with healthy ones (21 cases), which makes 21% and 9,5% respectively. On the contrary, allele G was more common in healthy people (90.5%) of approximately the same age than in patients suffering from myocardial infarction (79%) (Table II). The statistically significant association between A and G alleles and myocardium infarction showed that G allele can be considered to be preventive factor for myocardium infarction and, conversely, minor A allele can be considered to be the risk factor (Table IV).

Thus, not only does the presence of A nucleotide instead of G in polymorphic locus increases the risk of myocardial infarction, but homozygosity for both alleles (that is for minor gomozygoous A/A). Such a risk for heterozygous G/A was not established because of the presence of the opponent allele (G). Therefore, when only analyzing the SNP without genotyping one gets both genotypes having polymorphic nucleotide A. Those are G/A and A/A wherein G/A genotype masks A nucleotide contribution.

David R. Crosslin and co-authors [12] established the same role of A allele in the risk of myocardial infarction for the other polymorphism of haplotype B in Europeans, wherein almost every test showed a significant association between myocardial infarction and A allele instead G allele in rs17222842.

The data on the role of rs17216473 polymorphism is few and contradictory; mostly they dispute the data we obtained. So, the absence of a statistically significant association between SNP rs17216473 ALOX5AP gene and myocardium infarction was shown by David R. Crosslin and co-authores [12]. Helgadottir, and co-authores (2005) [4] also did not establish the essential correlation between haplotype B and the risk of myocardial infarction in Scottish people but found such an association in a group of English people.

Haplotype B also did not correlate with myocardial infarction in some researches [7, 8] This study had been carried out on a large group within the Central European population) (Germany) [8].

However, in the most studies it was reported the said polymorphism contributed to ischemic heart disease. David R. Crosslin [12] showed the statistically significant association between the rs17216473 polymorphism of ALOX5AP gene and the early onset of ischemic heart disease. Linsel-Nitschke et al [10] confirmed the correlation between haploptype B and the increased risk of coronary artery disease in German patients. B haplotype is supposed to contribute to pathogenesis of coronary artery disease in Europeans [10].

Tsai and co-authors [11] established that the SG13S377 (rs17216473) polymorphism of B haplotype was greatly and statistically significantly associated with the risk of ischemic heart diseases. This connection was without reference to the other polymorphisms, age, sex and the level of cholesterin. On the contrary, Huang and co-authors [6] pointed at an absence of correlation between rs17216473 and ischemic heart disease having based on the meta-analysis of Chinese population. However, it is necessary to emphasize that Tsai and co-authors [11] examined the white Americans but Huang and co-authors [6] examined Chinese. It is quite clear that Ukrainians are genetically closer to white Americans than to Chinese.

The role of the rs17216473 polymorphism of the ALOX-5AP gene in the myocardial infarction development in the Ukrainian population has been studied for the first time, the association between genotype A/A, allele A and the risk of myocardial infarction onset in Ukrainian population has been shown for the first time. Moreover, in the course of this study, humans-homozygotes with the minor allele A (A/A) were identified in the Ukrainian population for the first time. All of them had suffered from myocardial infarction. In the control group (healthy at about the same age), humans-homozygotes with the minor allele A were not found.

Consequently, all carriers of A/A genotype are proposed to belong to the group with an essential risk of myocardial infarction onset in Ukrainian population wherein the carri-

ers of G/G genotype are considered to belong to the minor risk of this disease onset, the carriers of G/A genotype have the medium risk of myocardial infarction onset.

The data we received may in the future be of interest to preventive medicine, for example, minimizing aggressive factors such as smoking, stress, unhealthy diet for people-carriers monozygotes A / A of the specified polymorphism. On the other hand, the data from our study may be further used for professional selection in activities that involve excessive stress, such as in the Armed Forces or in the police.

Surely, SNP in locus rs17216473 of ALOX5AP can`t be the only polymorphism that contributes to cardiovascular diseases and myocardial infarction in particular. In the recent study, which had performed in the Chinese population, the LTA4-H rs2540487 genotype was associated with the risk of myocardial infarction in overdominant model, wherein no associations of ALOX-5AP rs10507391, LTA4-H rs2072512 or rs2540477 and myocardial infarction were observed [15]. Some polymorphyns of ALOX5 gene were shown to associate with the reduced risk of stroke, exactly the rs3740107 in the recessive model in a Chinese Han population [16] and the GG genotype of rs2029253 [17]. The carriers of C allele in rs730012 of *LTA4H* gene and the rs6538697 CC genotype of *LTA4H* gene had an increased risk of ischemic stroke [17].

Beyond all doubt, the SNPs` particication in cardiovascular diseases needs the further study.

## CONCLUSIONS

1. The polymorphism rs17216473 (SG13S377) of gene encoding ALOX5AP has been established to be statistically significantly associated with the risk of myocardial infarction in Ukrainian population.
2. A/A genotype has been shown to contribute to myocardium infarction and on the contrary, G/G has been associated with the absence of it. G/A genotype has not been statistically significantly associated with myocardial infarction.
3. It has been established the statistically significant connection between the risk of myocardial infarction and the presence of allele A (minor allele) of said polymorphism. Allele G, vice-a-versa, has been statistically significantly linked to the absence of myocardial infarction.
4. All humans-homozygotes with the minor allele A (A/A) had suffered from myocardial infarction.

Summarizing our obtained results, we assume the carriers of G/G genotype to have a minimal risk of myocardial infarction onset, the carriers of G/A genotype to have a moderate risk and the carriers of A/A to have a great risk.

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ORIGINAL ARTICLE

# THERAPEUTIC APPROACHES TO THE CORRECTION OF COGNITIVE IMPAIRMENT IN PATIENTS WITH HYPERTENSION AND TYPE 2 DIABETES

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## ABSTRACT

**The aim:** Was to improve the effectiveness of the treatment of cognitive impairment in patients with hypertension and type 2 diabetes.

**Materials and methods:** 56 patients (11 women and 45 men, average age 61.7 ± 4.3 years) with hypertension II, 2 degree and type 2 diabetes (average severity, subcompensation stage) were examined. 40 patients had moderate CI and 16 had mild CI. After the examination, the patients were divided into two groups and treated accordingly. The control group consisted of 20 healthy individuals.

**Results:** Vascular brain lesions that cause hypertension and diabetes very often lead to impaired cognitive function whose therapeutic correction has received little attention, especially in the pre-operative stages. 56 patients have been examined to study the efficacy and safety a combination of Phenibut and Ipidacrine as an additional therapy to standard basic treatment (antihypertensive and hypoglycemic) for the correction of cognitive dysfunction in patients with comorbidity of hypertension and type 2 diabetes mellitus.

**Conclusions:** One month after the beginning of the treatment, an improvement in psycho-emotional state and psychometric parameters was identified, which was manifested by an increase in concentration of attention, memory, psychomotor functions, speech activity together with normalization of blood pressure and metabolic parameters.

**KEY WORDS:** hypertension, diabetes mellitus, cognitive impairment

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## INTRODUCTION

Arterial hypertension (AH) and diabetes mellitus (DM) of 2 types are among the three most common noncommunicable diseases and, also, are risk factors for cardiovascular complications, which are leading in the world as for the frequency of fatal cases [1]. Prolonged existing uncontrolled hypertension leads to the secondary damage to the heart and blood vessels and almost all vessels in the body from the aorta to the capillaries suffer from it. The lesion of the brain as a target organ of AH is manifested not only by strokes but also by impaired cognitive functions (memory, thinking, attention and so on). Vascular cognitive disorders are disorders of higher brain function caused by cerebrovascular pathology. This concept combines both vascular dementia and less severe cognitive impairment of vascular etiology [2]. Recently, the problem of cognitive disorders is becoming more and more of a medical and socio-economic importance, which is associated with an increase in the life expectancy of economically developed countries and the one in the prevalence of both moderate cognitive disorders and dementia [3].

Currently, in a large number of patients of working age with insufficiently controlled blood pressure (BP), even on the basis of antihypertensive therapy, cognitive functions have not been sufficiently studied [4,5]. Moreover, in the specified cohort of patients, asymptomatic lesions of cerebral vessels are more common among other target organ lesions [6]. Therefore, it is necessary to diagnose cognitive impairment as early as it is possible, i.e. before the onset of pronounced clinical signs, in order to optimize the tactics of managing such patients.

It is known that the treatment of hypertension in patients with type 2 diabetes is a complex problem, often with unsatisfactory results, which necessitates the search for new, effective ways of treating this pathology. In addition, when prescribing antihypertensive therapy to patients with hypertension and type 2 diabetes it is necessary to take into account their effect on the functional state of target organs, including cognitive function, as well as their effect on lipid and carbohydrate metabolism [7]. The use of a combination of an ACE inhibitor and a calcium antagonist amlodipine has shown to significantly affect the elastic properties of blood vessels in patients with hypertension and with type 2 diabetes mellitus [8], but the effect of this therapy on cognitive function in patients with hypertension type 2 diabetes with cognitive impairment remains unclear.

In our country, drugs with neurometabolic effects are used for treatment of mild and moderate cognitive impairments. The presence of the neuroprotective properties in a drug is fundamental, as it is also about the prevention of the growth of cognitive disorders and the development of dementia. In this regard, Phenibut, a representative of the

modern generation of nootropic agents with GABAergic activity gains lots of interest. Anxiolytic, nootropic, anti-stress and tranquilizing effects of Phenibut are realized due to the influence of GABA on the functional state of the nervous and cardiovascular systems [9]. It has been demonstrated under the influence of phenibut, that the drug stimulates learning and memory improvement, increases physical performance, eliminates psycho-emotional tension, anxiety, fear and improves sleep [10]. At the same time, unlike tranquilizers, indicators of higher nervous activity such as attention, memory, speed and accuracy of sensory-motor reactions are improved under the influence of Phenibut [10].

Ipidacrine exhibits the following pharmacological effects: restores and stimulates neuromuscular transmission; restores the impulses in the central and peripheral nervous systems; enhances contractility of smooth muscle organs under the influence of all antagonists except potassium chloride; improves memory and learning abilities; inhibits the progressive development of dementia; specifically stimulates the CNS; exhibits analgesic effect; exhibits antiarrhythmic effect [11]. The combination of Phenibut and ipidacrine properties causes an increase of the cognitive functions of the brain due to the influence on the GABAergic, cholinergic and dopaminergic brain systems.

Anti-stress drugs have not yet been used in the comorbidity of hypertension and type 2 diabetes in widespread clinical practice. The effect of neuroprotective therapy on the state of the stress system in patients with hypertension and type 2 diabetes has not been sufficiently studied. The research based on a systematic approach methodology will allow to develop a strategy for optimizing the activity of a multilevel stress system taking into account the individual characteristics of patients with cognitive impairment. This research seems promising and justified for patients with hypertension in combination with type 2 diabetes

## THE AIM

The purpose of the study was to improve the effectiveness of the treatment of cognitive impairment in patients with hypertension and type 2 diabetes.

## MATERIALS AND METHODS

56 patients (11 women and 45 men, average age 61.7 ± 4.3 years) with hypertension II, 2 degree and type 2 diabetes (average severity, subcompensation stage) were examined. 40 patients had moderate CI and 16 had mild CI. The diagnosis of hypertension was established in accordance with the recommendations of the European Society of hypertension and the European Society of Cardiologists (ESH / ESC, 2013) [12]. The diagnosis of type 2 diabetes was established according to the general recommendations of the European Association for the Study of Diabetes (EASD, 2013) [13].

The diagnosis of moderate cognitive impairment (MCI) was established according to the criteria of R.S. Petersen et al. [14]. The diagnosis of mild CI was established in accordance with the criteria of N.N. Yahno et al. [15]. All patients were diagnosed with at least one of the following complaints: noise, tinnitus, decreased performance, headache, dizziness, instability in walking, emotional lability as well as sleep disorders.

All patients underwent general clinical examination, physical examination, measured office blood pressure, heart rate (HR), total blood and urine analysis, biochemical blood test with determining of fasting serum glucose (GCN) levels, glycosylated hemoglobin (HbA1c) levels in whole blood, of insulin and lipid profile; insulin resistance was evaluated by the NOMA-IR index.

Daily blood pressure monitoring (DMBP) was performed using an «ABPM-02» device (Meditech, Hungary). The following indicators were determined: daytime, nighttime, average daily (24 hours) SBP and DBP as well as heart rate [16].

Expanded neuropsychological research, which included a set of quantitative tests for attention, memory, psychomotor functions, intellectual abilities, language activity, etc. were performed by a neurologist. The study of cognitive functions was performed according to the method of A.R. Luria [17], the short scale of the study of mental status (MMSE) [18] and the Montreal scale of assessment of cognitive functions (MoCa) [19].

After registration of baseline data, 28 patients of the main group (group 1) were prescribed basic therapy for hypertension (lisinopril 10-20 mg / day, amlodipine 10-20 mg / day) and a fixed combination of Phenibut 300 mg and Ipidacrine hydrochloride 5 mg («Kognifen», « Olainpharm", Latvia) 1 capsule 3 times a day for 4 weeks. 28 people who were in the comparison group (group 2) were prescribed basic AH therapy. Patients in both groups also received antihypertensive therapy (metformin + gliclazide), statins, antiplatelet therapy. These patient groups were compared according to the age and gender. The control group consisted of 20 healthy individuals (4 women and 16 men, average age 60.8 ± 2.9 years).

All patients successfully completed the study according to the protocol. The second study was performed after 4 weeks of treatment. Side effects and undesirable effects during this period have not been reported.

The mathematical computer processing of the study results was carried out using the software package «Statistica 8.0» (StatSoft Inc, USA). Mean value (M), variance, standard deviation, median (m), probability and significance leveli (p) have been calculated. Differences were considered significant at the level of statistical significance $p < 0.05$. The method of correlation analysis with the calculation of the Pearson correlation coefficients (with normal distribution) and Spirman (with a distribution different from normal) were used in order to evaluate the relationship between the indicators.

## RESULTS

The results of numerous clinical studies [20-24] indicate that hypertension increases the risk of cognitive impair-

**Table I.** Indicators of blood pressure according to the office measurement and DMBP of patients with hypertension

| Indicators | 1st group (n=28) | | 2nd group (n=28) | |
|---|---|---|---|---|
| | before therapy | after therapy | before therapy | after therapy |
| Sphygmomanometry: | | | | |
| SBP, mmHg | 164,3±6,3 | 132,5±3,5*** | 165,1 ± 6,4 | 135,8 ± 3,5*** |
| DBP, mmHg | 93,8 ± 4,5 | 80,3 ± 3,6* | 94,1 ± 4,6 | 82,2 ± 3,8* |
| DMBP | | | | |
| SBP24, mmHg | 151,6± 4,6 | 128,5 ± 4,3*** | 151,9 ± 4,5 | 129,3 ± 4,1*** |
| DBP24,mmHg | 91,7 ± 4,2 | 78,3 ± 3,5** | 91,6 ± 4,3 | 79,1 ± 3,3** |
| PBP, мм рт.ст. | 57,9 ±2,3 | 50,1 ± 2,3* | 57,7 ±2,5 | 50,8 ± 2,1* |
| TI SBPday, % | 61,5 ± 7,3 | 35,1 ± 7,1** | 62,1 ± 7,4 | 36,3 ± 7,1** |
| TI SBPnight, % | 65,1 ± 5,3 | 49,7 ± 4,1* | 66,3 ± 5,1 | 49,5 ± 4,3* |
| TI DBPnight, % | 43,3 ± 5,1 | 20,9 ± 4,3*** | 43,2 ± 5,3 | 21,3 ± 4,1*** |

Notes:
1. * - reliability of differences compared to the original data;
2. * - p <0.05;
3. ** - p <0.01;
4. *** - p <0.001.

**Table II.** Indicators of blood pressure (office and according to DMBP) patients with hypertension and type 2 diabetes.

| Indicators | 1st group (n=28) | | 2nd group (n=28) | |
|---|---|---|---|---|
| | before therapy | after therapy | | before therapy |
| TC, mmol / l | 6,31 ± 0,35 | 5,23 ± 0,28* | 6,35 ± 0,32 | 5,29 ± 0,25* |
| LDL-C, mmol / l | 2,56 ± 0,12 | 2,15 ± 0,10* | 2,57 ± 0,11 | 2,17 ± 0,10* |
| HDL-C, mmol / l | 0,98 ± 0,06 | 1,13 ± 0,02 | 0,95 ± 0,05 | 1,15 ± 0,03 |
| Tg, mmol / l | 2,28 ± 0,07 | 1,89 ± 0,06 | 2,35 ± 0,07 | 1,98 ± 0,05 |
| FBG, mmol / l | 7,30 ± 0,35 | 6,28 ± 0,31* | 7,31 ± 0,36 | 6,29 ± 0,30* |
| HbA1c, % | 7,19 ± 0,42 | 6,13 ± 0,40* | 7,21 ± 0,42 | 6,36 ± 0,31* |
| Insulin, uU / ml | 22,17 ± 0,49 | 19,09 ± 0,45 | 22,21 ± 0,46 | 19,08 ± 0,45 |
| HOMA-IR | 7,21 ± 0,56 | 5,42 ± 0,52* | 7,23 ± 0,59 | 5,46 ± 0,47* |

Notes:
1. * - reliability of differences compared to the original data;
2. * - p <0.05.

ment. It has been shown that the frequency of cognitive dysfunction increases with type 2 of diabetes mellitus [25, 26]. The exact mechanisms forming the basis of the association between type 2 diabetes and dementia are not clear. However, the effect of type 2 diabetes on cognitive function is probably realized through a whole set of mechanisms, reflecting the metabolic complexity of this disease. At the same time, studies on the characteristics of cognitive impairment in comorbidity and type 2 diabetes mellitus as well as methods for their correction are insufficient.

It has been proven that a significant reduction of the risk of developing ischemic and especially hemorrhagic strokes can be achieved in the treatment of hypertension [27]. Moreover, lowering blood pressure has been shown to help improve the performance of screening tests for dementia and memory, which indicates the beneficial effect of antihypertensive therapy on cerebrovascular morbidity. At

**Table III.** Dynamics of indicators of neuropsychological testing of patients with hypertension and type 2 diabetes.

| Tests | Period of study | 1st group | 2nd group | Control group |
|---|---|---|---|---|
| | | (n=28) | (n=28) | |
| MMSE scale, | before therapy | 26,6±0,3 | 26,5±0,5 | 30±0,3 |
| score | after therapy | 29,1±0,2*** | 27,3±0,3 | |
| 10-word memorization test (word count) | before therapy | 6,0±1,1 | 6,1±1,7 | 8,7±0,2 |
| | after therapy | 8,5±0,2* | 7,0±1,5 | |
| MoCa, score | before therapy | 23,1±0,2 | 23,6±0,3 | 29,3±0,2 |
| | after therapy | 29,2±0,3*** | 24,1±0,3 | |

Notes:
1. * - reliability of differences compared to the original data;
2. * - $p < 0.05$;
3. *** - $p < 0.001$.

the same time, some of the cognitive functions (perception process, learning ability) may not improve against the background of a decrease in blood pressure, which indicates an uneven effect of a decrease in blood pressure on different cognitive functions [28,29].

In our study there was a significant decrease in blood pressure according to the office measurement of blood pressure and DMBP after a course of treatment the patients with comorbidity of hypertension and type 2 diabetes (table.1. Indicators of blood pressure according to the office measurement and DMBP of patients with hypertension). However, differences in the dynamics of BP between the groups were not observed.

To date, type 2 diabetes pharmacotherapy is highly effective in reaching targeted glycemic levels as well as additional positive effects, significantly reducing the risk of complications, possibly including cognitive impairment.

There was an improvement in carbohydrate metabolism, a decrease in the insulin resistance index of HOMA and a lipidogram in patients in both groups after the treatment.

## DISCUSSION

Drugs with neurometabolic effects are used in the treatment of mild and moderate cognitive impairment in Ukraine. The presence of the neuroprotective properties in a drug is fundamental, as it is also about the prevention of the growth of cognitive disorders and the development of dementia. In this regard, Phenibut, a representative of the modern generation of nootropic agents with GABA-ergic action is of a great interest. Due to the influence of GABA on the functional state of the nervous and cardiovascular systems, anxiolytic, nootropic, antistress, and tranquilizing effects of Phenibut are realized [30]. The drug has been demonstrated to improve memory and learning, increases physical performance, eliminates psycho-emotional tension, anxiety, fear and improves sleep [31]. However, unlike tranquilizers, indicators of higher nervous activity such as attention, memory, speed and accuracy of sensory-motor reactions are improved under the influence of Phenibut [32].

We have found in our study that with the same decrease in blood pressure and positive dynamics of glucometabolic indicators the additional appointment of a fixed combination of Phenibut and Ipidacrine hydrochloride in the complex therapy of patients with hypertension and type 2 diabetes led to a more significant subjective improvement ($p < 0.05$) according to the dynamics of complaints (from 17.5 [16.2; 19.0] at baseline to 8.6 [6.3; 9.1] after the treatment ($p < 0.05$) versus 17.5 [16, 3; 19.1] to 12.5 [10.9; 14.6] in the comparison group ($p > 0.05$).

Changes in neuropsychological testing during treatment are presented in table 3. According to the MMSE scale, after 4 weeks of drug therapy, statistically significant positive dynamics were found, which differed significantly in the group receiving an additional fixed combination of Phenibut and Ipidacrine hydrochloride. The same tendency was found in the 10-word memorization test. The MoCa scores make it possible to evaluate cognitive functions more accurately. Improved cognitive function after 4 weeks of therapy was observed, It occured more pronounced in group 1 which indicates an improvement in brain functionality under the influence of treatment with the addition of a fixed combination of Phenibut and Ipidacrine hydrochloride.

## CONCLUSIONS

Thus, the addition of a fixed combination of Phenibut and Ipidacrine hydrochloride to basic antihypertensive therapy increases the effectiveness of cognitive function and, mareover, along with good safety, manifests itself in improving the psychometric parameters of patients with hypertension and diabetes mellitus type 2.

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**Conflict of interest:**

*The Authors declare no conflict of interest.*


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ORIGINAL ARTICLE

# FEATURES OF CEREBROVASCULAR REACTIVITY IN PATIENTS OF YOUNG AGE WITH MIGRAINE

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## ABSTRACT

**The aim** of the study was to Doppler sonography study of cerebrovascular reactivity in young patients with migraine and comparison of autoregulation patterns between groups of migraine patients with aura and migraine without aura.

**Materials and methods:** We conducted the clinical Doppler examination of 124 young patients (18-45 years old), including 55 men and 69 women in the conditions of the clinical base of the Kharkiv Medical Academy of Postgraduate Education in 2017-2019. The criteria for involvement of patients in the study were: migraine without aura (group 1-63 patients), migraine with aura (group 2-61 patients) The control group consisted of 45 patients of the corresponding gender and age. The indicators of CVR were studied using the ultrasound device.

**Results:** A hyperreactive response to tests with CO2 and photoreactivity were more pronounced in the patients with migraine without aura. The patients with migraine with aura showed hyperreactivity in the test with O2, which was an indicator of the tendency to hyperconstriction. The hyperreactivity in the functional nitroglycerin test and the carotid compression test shows the state of the myogenic mechanism of changes in vascular tone. Insignificant hyperreactivity to orthostatic load detected in both groups shows the interest of the neurogenic regulatory link.

**Conclusions:** 1. The most important hemodynamic patterns in the patients with migraine are excessive perfusion with migraine without aura and difficult perfusion with migraine with aura.

2. For the patients with migraine without aura, a characteristic criterion for autoregulation is the pattern of hyperreactivity hypercapnic load and photostimulation.

3. The most important difference in the autoregulatory response in the patients with migraine with aura compared with migraine without aura is the hyperresponse to hyperventilation load, compression carotid and nitroglycerin tests.

**KEY WORDS:** migraine, Doppler sonography, cerebral hemodynamics, cerebrovascular reactivity

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## INTRODUCTION

Currently, migraine is widespread in the world and can reach 22% in women and 16% in men [1].

The problem of migraine is of interest due to its wide prevalence, growth of the incidence rate in people of young productive age, as well as diagnostic and therapeutic difficulties. Migraine is the primary neurogenic cerebral dysfunction with the presence of genetically determined stem failure, severe cortical hyperactivity with periodically occurring hypothalamus dysfunctions [2-3].

The method of transcranial dopplerography (TCD) of the major vessels of the head has been successfully used to diagnose lesions of extracranial and intracranial sections of the major arteries. Recently, a large number of works have been published on the study of cerebral hemodynamic disorders in patients with migraine with and without aura, during the attack-free period and during the attack [4-6]. Their results are very contradictory. The studies during the attack-free period did not show significant differences in average blood flow velocity in extra- and intracranial arteries in patients with migraine relative to the group of healthy subjects [7]. Cerebrovascular reactivity (CVR) is considered as an integral indicator of the adaptive capabilities of the cerebral circulatory system, the ability of the brain vessels to respond to changing functioning conditions and to optimize blood flow in accordance with these conditions. The use of functional loads in order to assess the reactivity of cerebral vessels makes it possible to objectify the activities of regulatory mechanisms that control cerebral circulation and ensure its functional stability. The use of TCD to assess CVR is informative in patients with migraine. Studies have shown increased reactivity of cerebral arteries to respiratory retention, hyperventilation, visual stimulation, and usage of glyceryl trinitrate (GTN) [8-9]. The study of patients with migraine without aura demonstrated hyperreactivity for hypercapnia, which returned to normal state after preventive treatment [10]. This demonstrates that cerebrovascular reactivity can be a marker of migraine severity. The high sensitivity of cerebral arteries to GTN has also been proven, which draws the particular interest to the mechanisms of nitric oxide (NO) transformation in migraine studies [7]. This theory

**Tabl I.** Clinical characteristics of migrainous attacks in patients.

| | Groupe 1 (n=63) | Groupe 2 (n=61) |
|---|---|---|
| Age, years | 36,2 ± 5,3 | 28,9 ± 7,1 |
| Duration of attacks, years | 13,8 ± 5,4 | 10,5 ± 6,1 |
| Frequenc of attacks, days/month | 5,8 ± 3,1 | 5,2 ± 2,7 |
| Average duration of attacks, h | 43,8 ± 11,7 | 57,6 ±12,2 |
| Intensity of the pain syndrome (VAS) | 6,9 ± 1,6 | 8,2 ± 1,4 |
| MIDAS (The degree of decrease in the functional activity of the patient with migraine) | 29,3 ± 7,6 | 33,8 ± 9,1 |

is supported by the study showing the initiation of the migraine attack by GTN infusion [7].

The combined use of loads reflecting the functioning of various circuits of vascular regulation (humoral metabolic, myogenic, neurogenic) in patients with migraine is of interest.

## THE AIM

Was to Doppler sonography study of cerebrovascular reactivity in young patients with migraine and comparison of autoregulation patterns between groups of migraine patients with aura and migraine without aura.

## MATERIALS AND METHODS

We conducted the clinical Doppler examination of 124 young patients (18-45 years old), including 55 men and 69 women in the conditions of the clinical base of the Kharkiv Medical Academy of Postgraduate Education in 2017-2019. The criteria for involvement of patients in the study were: migraine without aura (group 1-63 patients), migraine with aura (group 2-61 patients) in accordance with the criteria for the international classification of headache disorders (ICHD-3, 2018) [11]. The exclusion criteria were the presence of occlusions and hemodynamically significant stenoses of brain magistral arteries (BMA). All patients underwent clinical and neurological examinations. The intensity of the cephalgic syndrome was assessed using a visual analogue scale (VAS) and a headache diary filled in by the patient himself. (Tabl.I).

The state of blood flow in cerebral arteries and indicators of cerebrovascular reactivity (CVR) were studied using Ultima PA ultrasound device (RADMIR, Ukraine) and Angiodin transcranial Doppler apparatus (BIOSS, Russia). The study was performed indicators of linear blood flow velocity (BFV) in middle cerebral (MCA), anterior cerebral (ACA), posterior cerebral (PCA) arteries , siphons of internal carotid arteries, intracranial segments of vertebral arteries (VA), basilar arteries (BA), and the reactivity coefficients to hypercapnic (CrCO2) and hyperventilation reactivity (CrO2), orthostatic (CrOL) and antiorthostatic (CrAOL) load, functional nitroglycerin (CrFNT) and functional metabolic (CrFMT) tests, and overshoot coefficient (OC) with carotid compression test and photoreactive coefficient (PRC) for photostimulation. The control group consisted of 45 patients of the corresponding gender and age. Statistical analysis and material processing were performed using the Statistic 6.0 software package. Differences recognized to be statistically significant at $P < 0.05$.

The study complies with the requirements of the Helsinki Declaration and is approved by the ethics commission of the Kharkiv Medical Academy of Graduate Education.

## RESULTS AND DISCUSSION

The most indicative were changes in hemodynamics in MCA, which were manifested by the pattern of excessive perfusion and were characterized by an increase in LBV in MCA in the patients of the 1st group (84.2 ± 10.4 cm/s, CG – 61.6 ± 7.3 cm/s, $p < 0.05$) and the pattern of difficult perfusion in the patients of the 2nd group (57.4 ± 6.8 cm/s, CG – 61.6 ± 7.3 cm/s). There is also a slight increase in flow rate indicators in ACA, PCA, VA, BA compared with CG (Fig.1).

The patients of the 1st group showed hyperreactivity to $CO_2$ and $O_2$ loads (1.39 ± 0.05 and 1.37 ± 0.04, respectively, CG – 1.28 ± 0.04; $p < 0.05$). The patients of the 2nd group showed pronounced O2 load hyperreactivity (0.51 ± 0.06, CG – 0.36 ± 0.03; $p<0.05$), which indicates the vasoconstrictive nature of vascular reactions. In both clinical groups, a slightly enhanced response to orthostatic load was noted (0.16 ± 0.04 and 0.18 ± 0.03, respectively, CG – 0.13 ± 0.03). Also, in the patients with migraine there was a significant increase in CrFNT indices, more pronounced in the subjects of the 2nd clinical group (0.21 ± 0.03 and 0.26 ± 0.04, respectively; CG – 0.16 ± 0.04; $p < 0.05$). The OC values were increased in both clinical groups, with a significant predominance in the patients of the 2nd group (1.58 ± 0.04; CG –1.4 ± 0.05; $p < 0.05$). In both clinical groups, the PRC values were significantly increased, these changes were more characteristic for the patients of the 1st group (1.39 ± 0.07 and 1.35 ± 0.05, respectively; CG –1.2 ± 0.05; $p < 0.05$). The response to FMT and GA as a whole did not differ from the normative indicators. Figs. 2, 3 show the CVR indices in the patients with migraine.

The most indicative were changes in hemodynamics in MCA, which were manifested by the pattern of excessive perfusion and were characterized by an increase in LBV in MCA in the patients of the 1st group and the pattern of difficult perfusion in the patients of the 2nd group. The studies have led to the conclusion that there are various options for autoregulatory response in the patients with migraine without aura and migraine with aura. Hyperreactivity along all control loops was common for the patients

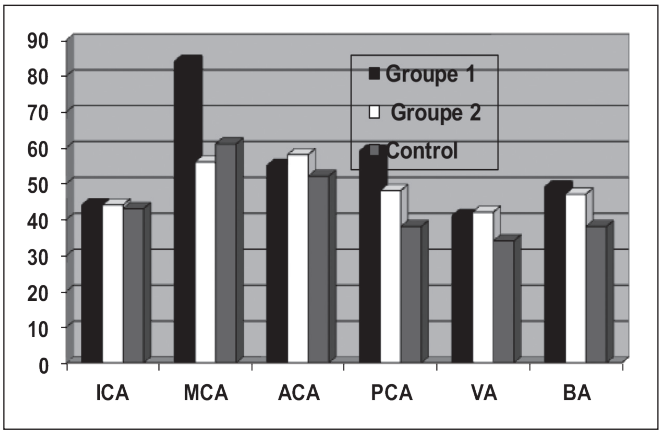


**Fig. 1.** The indicators of BFV in intracranial arteries in the patients with migraine

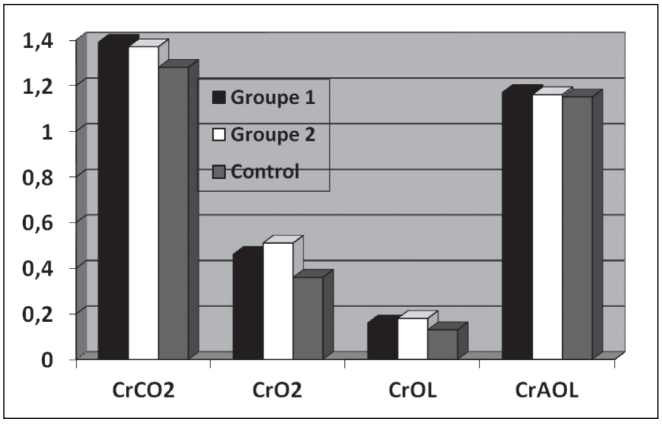


**Fig. 2.** The indicators of CrCO2, CrO2, CrOL and CrAOL in patients with migraine.

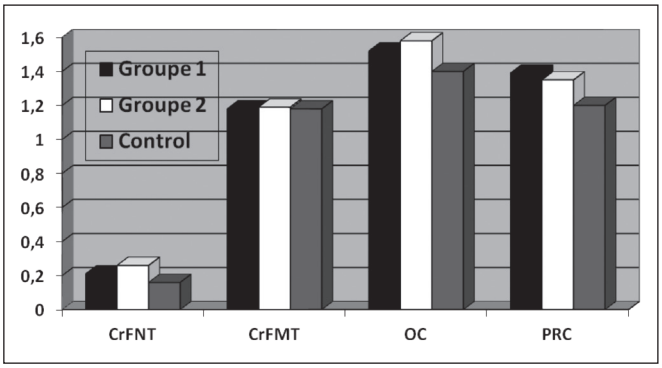


**Fig. 3.** The indicators of CrFNT, CrFMT, OC and PRC in patients with migrane.

of both groups. The most indicative was hyperreactivity to metabolic loads. A hyperreactive response to tests with CO2 and photoreactivity were more pronounced in the patients with migraine without aura. The patients with migraine with aura showed hyperreactivity in the test with O2, which was an indicator of the tendency to hyperconstriction. The confirmation of this statement was also the hyperreactivity to the carotid compression test (overshoot coefficient), which is the reflection of the hypertonicity of resistive vessels. We also see a similar answer when analyzing nitroglycerin test parameters, which shows the state of the myogenic mechanism of changes in vascular tone. Insignificant hyperreactivity to orthostatic load detected in both groups shows the interest of the neurogenic regulatory link. The neurogenic regulatory loop is not leading in the patients with migraine, but is an indirect reflection of the presence of autonomic imbalance observed in most patients with migraine. Thus, a commonality of responses of the patients of both groups to the majority of presented stimuli is observed, the differences are in the presence of vasoconstrictor reactions and background hypertonicity of resistive vessels in th patients with migraine with aura.

Most previous studies of cerebrovascular reactivity in migraines [8-10]. were differential to patients with migraine with aura and without aura. Also, the studies reflected the state of certain contours of autoregulation (humoral-metabolic, myogenic, neurogenic) and did not give a holistic view of the state of vascular reactivity in a particular patient. For the first time, we have proposed the diagnostic algorithm for Doppler studies in migraine, which allows distinguishing cerebrovascular reactivity patterns, specific for groups of patients with migraine with aura and migraine without aura

## CONCLUSIONS

1. The most important hemodynamic patterns in the patients with migraine are excessive perfusion with migraine without aura and difficult perfusion with migraine with aura in the middle and posterior cerebral arteries.
2. For the patients with migraine without aura, a characteristic criterion for autoregulation is the pattern of hyperreactivity to most functional loads, more pronounced with hypercapnic load and photostimulation.
3. The most important difference in the autoregulatory response in the patients with migraine with aura compared with migraine without aura is the reactions associated with hyperconstriction of resistive vessels – a pronounced hyperresponse to hyperventilation load, compression carotid and nitroglycerin tests.
4. The indicators of cerebrovascular reactivity reflect the difference in the hemodynamic mechanisms of the migraine attacks in the patients with migraine with aura and without aura and can be used to clarify the diagnoses and individualize treatment in these patients.

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*The Authors declare no conflict of interest.*

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ORIGINAL ARTICLE

# SPECKLE TRACKING DOBUTAMINE STRESS ECHOCARDIOGRAPHY DIAGNOSTIC ACCURACY IN PRIMARY CORONARY ARTERIES DISEASE DIAGNOSIS

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## ABSTRACT

**The aim** of the work was to evaluate STE feasibility as DSE visualization method and its accuracy compared to coronary angiography (CAG) in the patients with moderate-to-high coronary arteries disease (CAD) risk.

**Materials and methods**: We prospectively examined 140 pts (84 (60.0%) men) with suspected CAD in order to verify diagnosis and evaluate myocardial viability and coronary reserve.

**Results**: Mean LV EF was 54.4±15.8%. All pts had normal BP and HR during the test. There were no significant hemodynamics alterations during the test. There were no significant complications during DSE – 15 (12.9%) cases of different relatively low-grade supraventricular and ventricular arrhythmia, mainly transitory without interventions. There were 116 (82.9%) positive DSE results, of which 2 (1.72%) were false-positive. In 2 (8.3%) pts with negative DSE results CAG revealed 1-vessel insignificant (50 – 70%) lesions with developed collaterals (false-negative results). According to DSE and CAG results, 96 (82.3%) pts underwent revascularization interventions – 86 (89.6%) PCI's and (10.4%) CABG surgeries.

Sensitivity and specificity of DSE with STE for primary CAD diagnosis according to "golden standard" CAG results were 98.3% and 91.7%, respectively, with identical positive and negative predictive value and very high method overall accuracy (AUC = 0.98) and OR = 627.0 ($p<0.0001$). Sensitivity and specificity of DSE with STE for defining indications for intervention and revascularization were 97.9% and 91.7%, respectively, with high overall accuracy (AUC = 0.95; OR = 564.0, $p<0.0001$). Combined quantification of ΔGLS and ΔWMSI for primary CAD diagnosis showed significantly lower sensitivity 86.2% ($p=0.0002$) and specificity 80.4% ($p=0.0064$) with significantly lower integral method accuracy (AUC 0.83, $p<0.0001$).

**Conclusions:** DSE with STE as a visualization method is a safe and optimal method for ischemia diagnosis and myocardial viability and coronary reserve evaluation in the pts with CAD suspicion. Given the lower ΔGLS and ΔWMSI accuracy compared to integral DSE with STE result evaluation, as well as frequent GLS growth in significant amount of patients with definite positive test result, authors recommend evaluating integral test result rather than strain value.

**KEY WORDS:** coronary heart disease, speckle tracking, stress echo

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## INTRODUCTION

Timely and urgent diagnosis of coronary arteries disease (CAD) and coronary artery territory plays the key role in managing patients (pts) in daily cardiology practice, including those having acute coronary syndromes (ACS) or revascularization procedures history, namely percutaneous (PCI) and surgeon venous and mammary grafts (CABG). Indications for revascularization is the main dilemma facing cardiologist and cardiovascular surgeon, while reliable ischemia verification and myocardial viability evaluation, especially in the pts with multivessel disease and ischemic cardiomyopathy (ICM) largely determines patient management tactics and scope of intervention. Prompt primary CAD diagnosis is also of big importance, especially in large centers with big pts flow.

Conventional routine echocardiography (EchoCG) is not reliable enough for CAD diagnosis expertise even with tissue Doppler imaging (TDI). Stress EchoCG with dobutamine (DSE) is an acknowledged today noninvasive method for such purposes with acceptable diagnostic accuracy. Still, visual regional contractility abnormalities evaluation during DSE remains rather subjective, since evaluation largely depends on operator's experience in acquiring images and pharmacological stress results interpretation [1,2].

Speckle tracking EchoCG in B-mode (STE) or myocardial deformation study is a widely spreading method for global and regional left (LV) and right (RV) ventricles function evaluation. STE provides important information about myocardial

strain by its quantification with those advantages over TDI that strain does not depend on angle between angle between ultrasound (US) beam and myocardial strain vectors during contraction or relaxation [1-4]. Today STE is being actively verified by many centers and used in diagnosis and treatment efficacy verification in many coronary and non-coronary myocardial diseases, having high sensitivity in detecting preclinical myocardial dysfunction even with normal results of traditional resting EchoCG in B-mode [1-7]. High diagnostic accuracy of STE was proven for verifying coronary territory of lesions with its high correlation with coronary angiography (CAG) results regarding levels of lesions in different CAD forms [8-11], including acute coronary syndrome (ACS) with or without ST segment elevation [12-15].

Strain indices do not so much depend on neighbor segments "translational" motion and injured segments "pulling-up" which may lead to misinterpreting of segments kinetics during qualitative visual evaluation of regional evaluation of segments during DSE. Today there are quite a lot of recent publications evaluating clinical application of STE during STE in different pts groups with CAD [2,7,8,10-12], allowing to view STE for DSE as a highly trustworthy quantitative method for ischemia diagnosis and myocardial viability evaluation, but they usually describe small pts cohorts. In our center, we performed about 500 DSE procedures using STE in various clinical situations, and may conclude it to become a routine clinical practice. Still, there were no evidence-based studies of the matter in our country.

## THE AIM

Aim of this study was to assess the diagnostic value of STE at rest and during DSE in primary CAD diagnosis and diagnostic accuracy of B-mode longitudinal strain evaluation as a method indicative of CAG and revascularization necessity.

## MATERIALS AND METHODS

We prospectively examined 140 pts (84 (60.0%) men and 56 (40.0%) women) aged 57.6±11.3 years with CAD suspicion admitted for diagnosis verification and defining indications for coronary interventions. All pts underwent DSE with longitudinal strain study as per STE. Exclusion criteria were significant valvular heart disease, history of any cardiac pacemaker implanted, and permanent atrial fibrillation (AF). Pts with complete left hemiblock were included into the study since our experience shows that strain dynamics during pharmacological stress is usually obvious in asynchronous segments, too. Amosov National Institute of Cardiovascular Surgery (NICVS) of NAMS of Ukraine local ethics committee approved study protocol. All patients gave written informed consent. Authors declare no conflicts of interest.

### IMAGING

All examinations were performed using Vivid E9 (General Electric, USA) ultrasound equipment with M5S-D probe. Images for strain studies were acquired in patients' left decubitus position from parasternal and apical windows (4-, 2-, and 3-chambers) with video records at rest and at every stage of pharmacological dobutamine stress, and at restitution. 2D greyscale videos were recorded at 60 – 70 Hz frame rate in order to optimize speckle-tracking quality with their sequential off-line analysis. Quantitative longitudinal strain analysis was performed using Vivid E9 software package.

### DOBUTAMINE STRESS ECHOCG

DSE was performed before CAG for primary ischemia diagnosis in coronary territories of interest.

All patients were asked to withdraw nitrates for at least 48 h before test. In the beginning, we also asked to withdraw beta-blockers (BB) 48 h before test, but in the process of our work and experience accumulation, we made a conclusion that BB withdrawal is not necessary for ischemia diagnosis and myocardial viability and coronary reserve estimation by means of ST for the following reasons. Despite their negative chronotropic and inotropic effects BB are unable to influence ischemic or non-ischemic myocardial deformation changes due to dobutamine stress significantly. In addition, abrupt BB withdrawal in the pts with confirmed CAD, heart arrhythmia or arterial hypertension (AH) is relatively contraindicated.

DSE was performed according to standard protocol [2,10,13,16]. Dobutamine infusion was infused via intravenous line in four 3-min stages with dobutamine dose increase: (5)-10-20-30-40 mcg/kg/min under heart rate (HR) and blood pressure (BP) control with every tree min. dose increase and consecutive analysis of regional strains changes on a "bull's eye" LV model. Giving additional atropine for achieving age-adjusted target HF was considered inappropriate in the pts with confirmed CAD and ACS history, since our experience shows that STE is sensible enough to ischemic strain changes even if target HR is not reached.

With experience accumulation, we concluded that primary ischemia diagnosis requires start dobutamine dose of 10 mcg/kg/min, since 5 mcg/kg/min is not enough to provoke ischemia where STE did not reveal it at rest.

Test was performed with present anesthesiologist with necessary equipment for possible complications management (Ambu bag, intubation equipment, and cardioverter-defibrillator). In the test beginning 12-lead ECG was registered and rest EchoCG was performed with recording of rest ("native") longitudinal strain pattern (bull's eye) and global longitudinal strain (GLS) quantification. After that bull's eye pattern was quantified and qualified after every 3 min of each following dose increase stage, and after 3 min of restitution. HR normalization was reached in 3 to 5 min after infusion stop and did not require additional BB administration.

Stress protocol termination criteria were as follows:

1) age-adjusted submaximal HR was achieved as per formula [220 – age (years)] x 0.85 [2,10];

**Table I.** Criteria for STE dynamics during DSE qualitative assessment

| Data at rest | Dynamics | Interpretation |
|---|---|---|
| Normal strain | Continuous growth or stable high / normal strain | Territory without ischemia |
| Normal strain | Continuous strain drop | Ischemia / low coronary reserve |
| Normal strain | Growth at low doses and drop at higher doses | Ischemia («two phased» answer) / insufficient coronary reserve |
| Normal strain | Drop at low doses and growth at higher doses | Ischemia with high / sufficient coronary reserve |
| Mild-to-moderate strain decrease | Continuous strain growth at all stages | Moderate ischemia of viable myocardium at rest with sufficient coronary reserve / non-ischemic cardiomyopathy |
| Moderate-to-severe strain decrease / inverted strain | Strain growth at different stress stages | Severe ischemia of viable myocardium at rest (hibernation), including postinfarction scar zone |
| Severe strain decrease/ inverted strain | Absent strain reaction to stress | Scar / unviable myocardium |

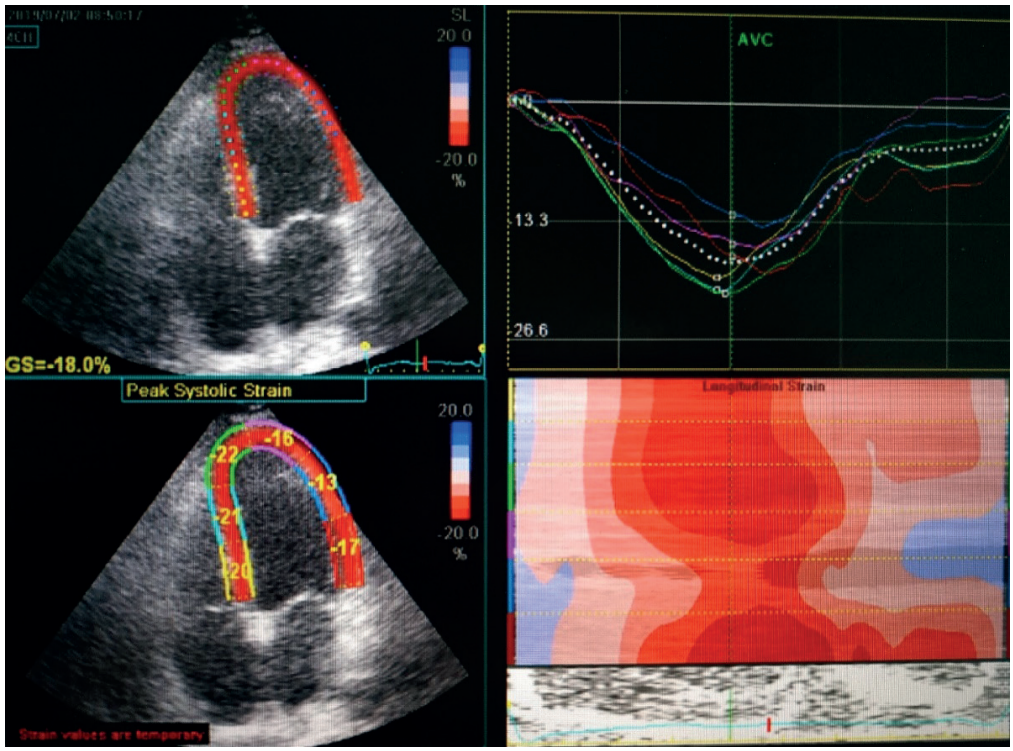


**Fig. 1.** Example of automatically plotted in time vs. strain graphs in apical 4-chamber (A4C) position in the patient with later approved moderate middle segment Cx lesion – decreased distal lateral segments strain.

2) signs of ischemia as per strain drop in neighbor 4 or more myocardial LV and RV segments;
3) patient reported intense angina-like pain;
4) ECG-signs of clinically relevant arrhythmia: atrial fibrillation (AF), "runs" of ventricular tachycardia (VT), ventricular bigeminy or trigemini or multiform or frequent premature ventricular complexes (VES), including pairs;
5) >2 adverse events of any other grade, including frequent premature supraventricular complexes (SVES), bradycardia or HR decrease compared to previous stress stages.

LV and RV kinetics were assessed by expert operators. 17-segment LV model was used [1,12,14,17]. Myocardial segments motion was graded according to kinetics type at each stage of stress as normo- (grade 1), hypo- (grade 2), a- (grade 3) or dyskinesia (grade 4). Any motion abnormality or deterioration by one or more grade was indicative of ischemia. Wall motion score index (WMSI) was calculated at rest and at peak stress stage for gradient ΔWMSI calculation.

## SPECKLE TRACKING ECHOCG

Longitudinal strain was assessed by 2D-STE as per standard protocol [1]. End-systole was identified by aortic valve (AV) closure. Operator traced endocardial borders with automated longitudinal strain quantification. Adequate tracking was verified in real time with correction, if necessary, by manual regions of interest adjustment. After tracking completion myocardial strain was automatically plotted in time vs. strain graphs for different cardiac cycle

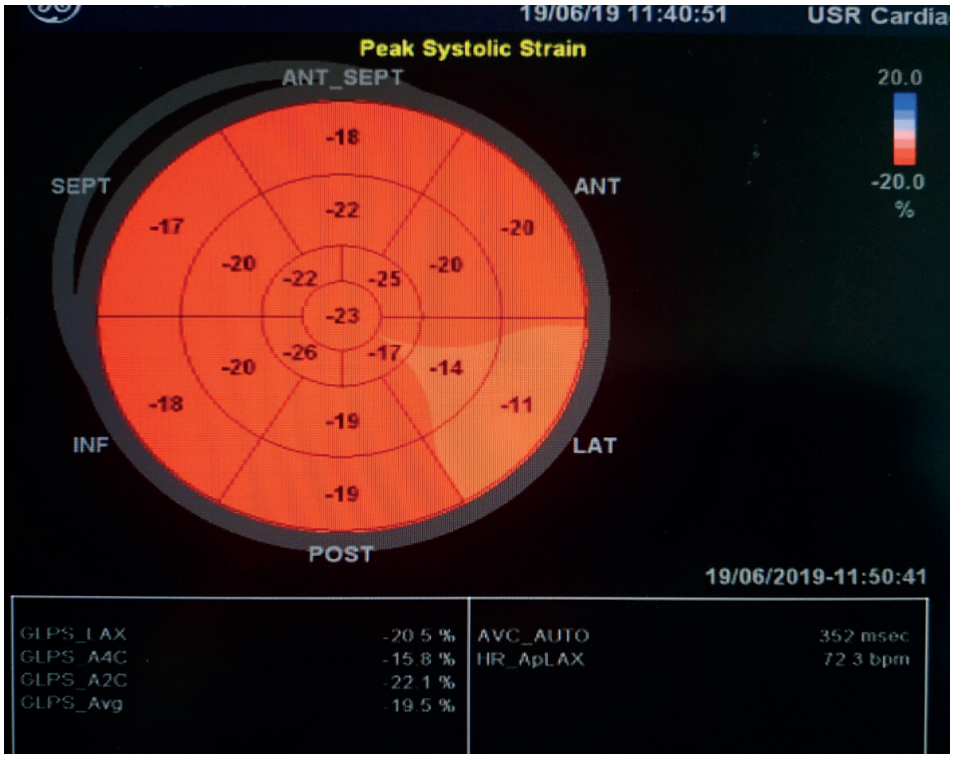


**Fig. 2.** 17-segments LV model (bull's eye): pattern of moderate Cx territory ischemia – moderate lateral-posterior longitudinal strain decrease with preserved global LV strain (GLS = – 19,5%).

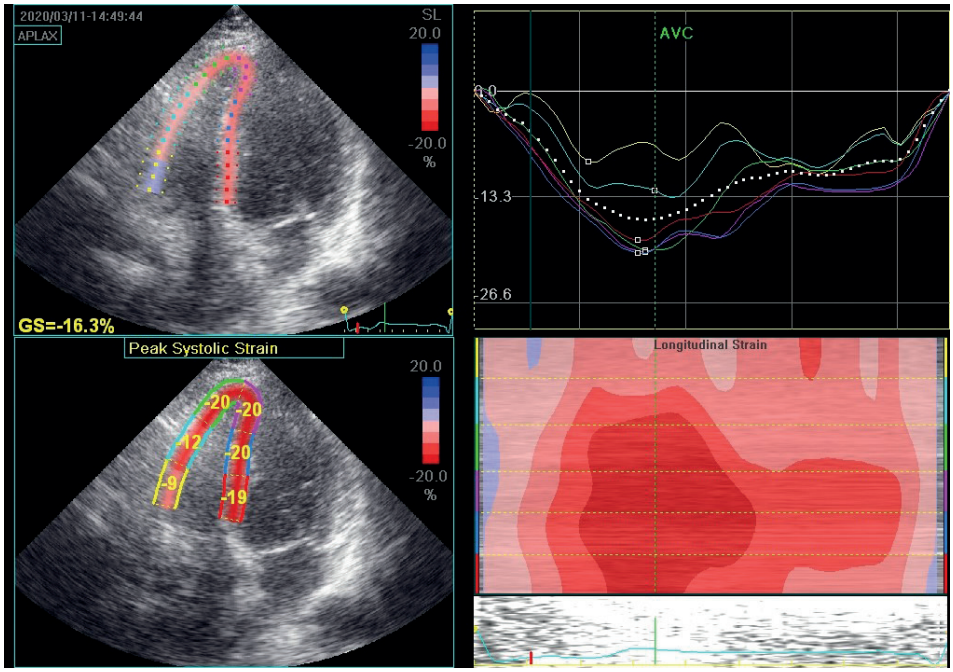


**Fig. 3.** Example of ischemia verification in RCA territory using RV strain quantification – moderate-to-severe basal and middle "free" strain drop (GLS= – 13.7%) with preserved IVS (LAD territory) strain (GLS = -19.7%).

phases identification (Fig. 1) and building the strain pattern on 17-segments bull's eye model (Fig. 2) after tracking was completed in all three apical 3-, 4- and 2-chambers positions. RV longitudinal strain quantification for "free" wall and interventricular septum (IVS) right side was electively performed for additional ischemia diagnosis verification in right coronary artery (RCA) territory (Fig. 3) identically to LV segments.

Dobutamine stress results were assessed in every separate coronary territory by evaluating longitudinal strain dynamics in the territories of left anterior descending (LAD) and circumflex (Cx) left coronary artery (LCA) branches, and RCA (Fig. 4). The absolute numerical GLS value was calculated at rest and at every stress stage with dynamics quantification ΔGLS. In addition, criteria of myocardial viability and coronary reserve were qualified (Table I).

## CORONARY ANGIOGRAPHY

All pts underwent CAG according to the Judkins technique after DSE. Images and video were assessed by expert operators with significant CAD definition of >70% luminary diameter stenosis.

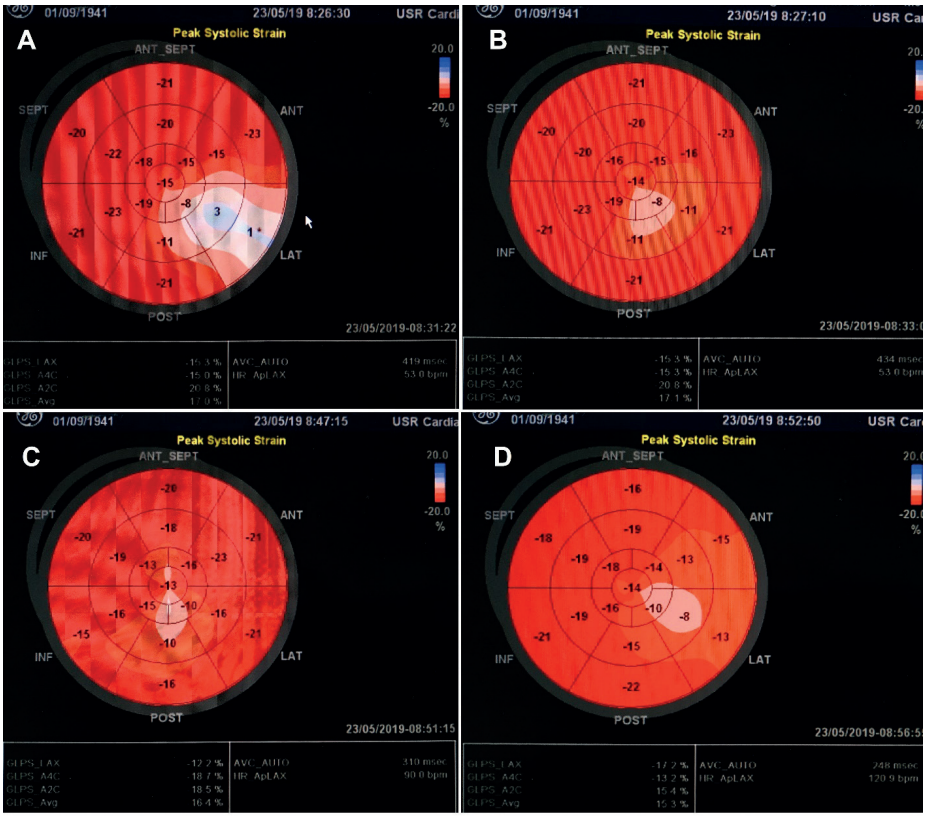


**Fig. 4.** A – D: continuous registration of strain patterns in bull's eye 17-segments model at rest and at 10, 20 and 40 mcg/kg/min dobutamine stress, respectively, in the patient with functional class 2 angina pectoris with ischemia in distal LAD territory (later proved by CAG). A: At rest, there is severe LV lateral wall strain decrease with local strain inversion. B: At 10 mcg/kg/min stress marked strain growth of previously compromised segments with preserved moderate decrease of apical anterior-lateral and posterior-lateral strains (ischemia with sufficient coronary reserve) without significant strains dynamics at the dose of 20 mcg/kg/min. C: Given targeted HR not reached (90'), test was prolonged to maximal stress. D: In 3 min. of 40 mcg/kg/min infusion there is a marked strain drop of whole antero-lateral LV wall and apex – moderate-to-severe ischemia ("two phased answer") without sufficient coronary reserve in LAD territory. Patient successfully underwent LCA LAD PCI by DES.

## STATISTICAL ANALYSIS

Data was analyzed using SPSS 20.0 software package for Windows (SPSS Inc., Chicago). Continuous variables are presented as mean ± SD, categorical – as percentages. In all cases p-value <0.05 was considered statistically significant. Method accuracy was evaluated by calculating sensitivity, specificity, positive and negative predictive values, and index of method prognostic ability "area under curve" (AUC). Intra- and interobserver variability were evaluated by repeated strain quantification and visual estimation of same video records within 10 – 14 days intervals and by two different independent operators in 10 randomly selected pts.

## RESULTS AND DISCUSSION

Mean pts age was 57.6±11.3 years (84 men – 60,0%) with mean LV ejection fraction (EF) 54.4±15.8%. AH with sustained medical treatment in history was in 118 (84.3%) pts. 38 (27.1%) pts had diabetes mellitus (DM) type II. 56 (40.0%) pts were tobacco smokers. High CAD risk according to SCORE charts was found in 121 (86.4%) pts, while compromised CAD family history was registered in 127 (90.7%) пациентов. In 10 (7.1%) pts there were various corrected or uncorrected congenital or valvular heart disease: 2 (1.4%) pts. after mitral (MV) and aortic (AV) valve replacement (MVR & AVR) due to rheumatic disease, 2 (1.4%) pts after AVR due to congenital bicuspid AV degeneration, 2 (1.4%) pts after congenital interatrial and interventricular septum correction (hemodynamically insignificant), and 4 (2.8%) pts with insignificant atherosclerotic AV disease. 22 (15.7%) pts had a history of different arrhythmia: 14 (10.0%) pts with paroxysmal AF, including 2 (1.4%) pts after radiofrequency ablation (RFA) without relapses, and 8 (5.7%) pts with various supraventricular (SVES) and ventricular (VES) low grade premature beats. Complete left hemiblock of unsure origin was diagnosed in 6 (4.3%) pts. 2 (1.4%) pts had DCM phenotype of unsure origin. All pts had normal BP and HR by the examination beginning (Table II).

There were no significant hemodynamics alterations during DSE. Maximal systolic (SBP) and diastolic (DBP) BP elevation at peak stress compared to rest values were 158.2±9.4 vs. 129.8±10.5 (p<0.0001) and 88.4±6.2 vs. 82.6±11.7 (p<0.0001) mm Hg, respectively. HR mean increase was 47.4±14.9' (113.8±23.8 vs. 66.4±8,1, p<0.0001). Age-adjusted submaximal HR was reached only in 72 (51.4%) pts, which was explained by the facts that: 1) a significant proportion of pts received BB due to AH and/or HF; 2) ischemia criteria were reached much earlier than HR was reached; 3) maximal dobutamine dose was seldom reached. BP and HR restitution in all pts was reached spontaneously in 3 – 5 min after dobutamine was stopped and required no medication correction. There was no significant difference between SBP, DBP and HR growth in the patients with positive and negative test results.

Maximal dobutamine dose constituted 26.6±10.3 mcg/kg/min., and majority of pts – 88 (62.9%) – reached maximal doe of 20 mcg/kg/min. In 2 (1.4%) pts test was stopped already at 10 mcg/kg/min stage due to marked ischemia signs by STE (severe strain decrease or inversion), while 50 (35.7%) pts made it to maximal stress of 40 mcg/kg/min,

**Table II.** Pts demographics in the study group

| Patients demographics | Total (n = 140) |
|---|---|
| Men, n (%) | 84 (60.0%) |
| Age, yeas | 57.6±11.3 |
| Arterial hypertension, n (%) | 118 (84.3%) |
| DM, type II, n (%) | 38 (27.1%) |
| Smoking, n (%) | 56 (40.0%) |
| LV EF, % | 54.4±15.8 |
| SBP, mm Hg | 129.8±10.5 |
| DBP, mm Hg | 82.6±11.7 |
| HR, beats/min. | 66.4±8.1 |
| High CAD risk, n (%) | 121 (86.4%) |
| CAD family history, n (%) | 127 (90.7%) |
| Valvular and congenital heart defects, including valvular replacement history, n (%) | 10 (7.1%) |
| Arrhythmia history, n (%) | 22 (15.7%) |
| Complete left hemiblock, n (%) | 6 (4.3%) |

mainly due to high coronary reserve (32 (22.9%) pts) or (18 (12.9%) pts) due to negative test result.

Pts with negative DSE result had no complications during or after procedure at all.

In the patients with positive DSE results, overall frequency of any complications during the study was only 15 (12.9%) cases. We had no cases of manifested angina pain, BP drop or VT paroxysms. In 4 (3.5%) pts AF paroxysm developed at submaximal or maximal dobutamine stress – in 2 (1.72%) cases cardioversion was used, while in 2 (1.72%) cases spontaneous sinus rhythm restoration took place in 10 – 15 min after dobutamine infusion stopped. In 6 (5.2%) pts there were solitary and group VES. 2 (1.72%) pts had frequent SVES, all hemodynamically insignificant and having stopped spontaneously in 5 – 10 min after infusion stop. 1 (0.86%) patient had relative HR decrease without manifested bradycardia. In 2 (1.72%) pts with history of AH and LV hypertrophy at the dose of 20 mcg/kg/min we found previously undiagnosed obstructive hypertrophic cardiomyopathy (HCM) with severe dynamic LV outflow tract obstruction (ΔP peak 127 and 135 мм Hg) (Table III and IV).

In 116 (82.9%) pts DSE result was positive, in 24 (17.1%) – negative. In the pts with positive DSE in 68 (58.6%) pts ischemia markers were present already at rest, and the following test was performed for myocardial viability and

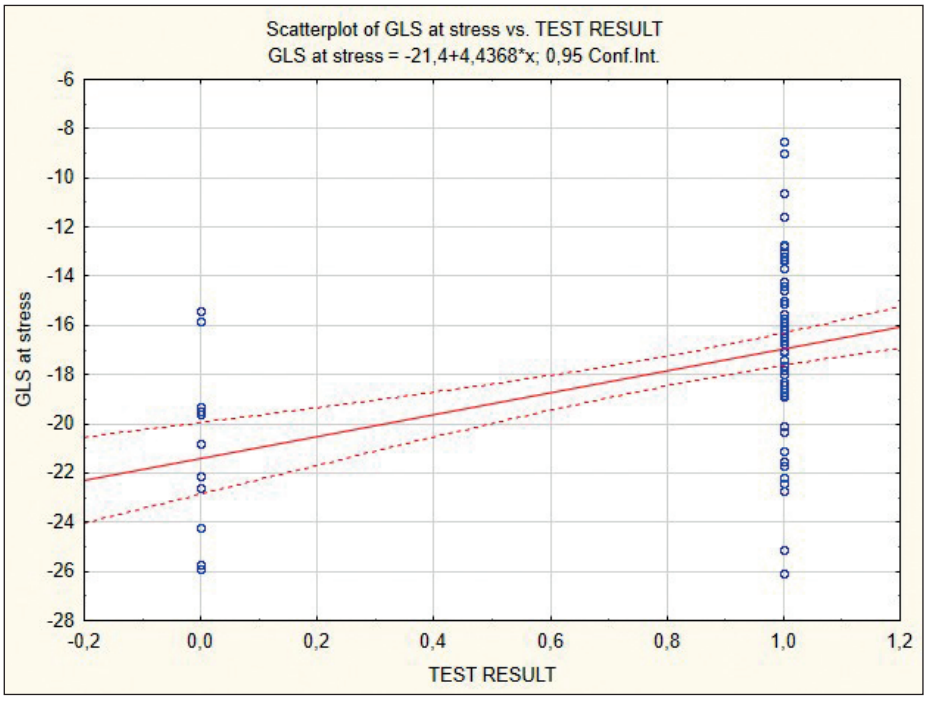


**Fig. 5.** Correlation between peak stress GLS value and general DSE test result (r = 0.43, p<0.0001).

**Table III.** Hemodynamics during DSE with STE and dobutamine stress dose in the study group

| Indices | Rest | Stress |
|---|---|---|
| SBP, mm Hg | 129.8±10.5 | 158.2±9.4, p<0.0001 |
| DBP, mm Hg | 82.6±11.7 | 88.4±6.2, p<0.0001 |
| HR, beats/min. | 66.4±8.1 | 113.8±23.8, p<0.0001 |
| Maximal dobutamine dose, mcg/kg/min. | 26.6±10.3 | |
| 10 mcg/kg/min, n (%) | 2 (1.4%) | |
| 20 mcg/kg/min, n (%) | 88 (62.9%) | |
| 40 mcg/kg/min, n (%) | 50 (35.7%) | |

**Table IV.** DSE with STE features and immediate results in the studied group

| DSE with STE results | |
|---|---|
| Positive, n (%) | 116 (82.9%) |
| Negative, n (%) | 24 (17.1%) |
| CAD diagnosis dobutamine dose, mcg/kg/min | |
| At rest | 68 (58.6%) |
| 10 mcg/kg/min. | 16 (13.8%) |
| 20 mcg/kg/min. | 26 (22.4%) |
| 40 mcg/kg/min. | 6 (5.2%) |
| Complications, n (%) | 15 (12.9%) |
| Angina pain, n (%) | 0 (%) |
| AF, n (%) | 4 (3.5%) |
| VT "runs", n (%) | 0 (%) |
| Frequent or multiform, n (%) | 6 (5.2%) |
| Frequent SVES, n (%) | 2 (1.72%) |
| Bradycardia or relative HR drop, n (%) | 1 (0.86%) |
| Previously undiagnosed HCM, n (%) | 2 (1.72%) |

coronary reserve evaluation in the territories of interest. In 16 (13.8%) pts ischemia markers were diagnosed at minimal dose of 10 mcg/kg/min, in 26 (22.4%) pts – at 20 mcg/kg/min, in 6 (5.2%) pts with high coronary reserve – only at 40 mcg/kg/min. As stated above, hemodynamically insignificant transitory complications were present only in the positive DSE group, of which only 2 (1,72%) cases required intervention (AF paroxysms cardioversion) (Table IV).

GLS dynamics in general studied group of insignificant with minor GLS -0.3% drop (p=0.51). In the pts with positive DSE result there was insignificant general -0.9% GLS drop 0.9% (p=0.055), while in the pts with negative DSE result GLS significantly grew – ΔGLS = +2.7 (p=0.015). However, we also found that regardless of general test result GLS dynamics could be either positive or negative in both groups. In positive DSE group on third of pts (n=38, 32.8%, p<0.0001) showed significant GLS growth (ΔGLS = +2.7, p=0.0032), while in two thirds GLS significantly dropped (ΔGLS = -2.7, p<0.0001). In negative DSE group majority of pts (n=22, 91.7%, p<0.0001) showed significant GLS growth (ΔGLS = +3.1, p=0.011), while its decrease in the minority of pts (n=2, 8.3%, p<0.0001) was insignificant (ΔGLS = -0,8, p=0.09) (Table V). With this, correlation between peak stress GLS and general test result was significant but weak (r = 0.43, p<0.0001) (Fig. 5).

All pts underwent CAG. In the pts with negative DSE result (24 (17.1%) pts) 2 (8.3%) cases of 1-vessel disease were diagnosed – 1 (4.15%) case of insignificant (50-60%) LAD lesion (patient with earlier undiagnosed HCM) and 1 (4.15%) case of insignificant (50 – 70%) RCA lesion with well-developed collaterals in both cases (false negative test result). Both cases were not stented. In the rest of cases, CAG showed intact coronary arteries (CA).

In the pts with positive DSE result (116 (82.9%) pts) there were 2 (1.7%) false-positive results with intact CA – both in the pts with long AH history and marked hypertensive "bull's eye" pattern at STE complicating results interpretation along with marked congenital CA tortuosity. Rest of cases showed different severity and prevalence of significant CA atherosclerotic lesions (Table VI).

Therefore, DSE with STE results sensitivity and specificity regarding primary CAD diagnosis compared to CAG results, as the "golden standard", were 98.3% and 91.7%, respectively, with identical positive and negative predictive values and very high general method accuracy (AUC = 0.98) and relative risk (OR = 627.0, p<0.0001).

**Table V.** GLS dynamics during DSE with STE

| | Rest | Peak stress |
|---|---|---|
| GLS in general group (n=140), % | -18.0±3.5 | -17.7±-4.0 |
| ΔGLS, % | - 0.3, p=0.51 | |
| GLS in positive DSE group (n=140), % | -17.9±3.5 | -17.0±3.6 |
| ΔGLS, % | - 0.9, p=0.055 | |
| GLS in negative DSE group (n=140), % | -18.7±3.8 | -21.4±3.6 |
| ΔGLS, % | 2.7, p=0.015 | |
| Positive DSE group (n=116) | | |
| Growth, n=38 (32.8%) | | |
| GLS, % | -16.1±3.5 | -18.8±4.2 |
| ΔGLS, % | 2.7, p=0.0032 | |
| Drop, n=78 (67.2%), p<0.0001 | | |
| GLS, % | -18.7±3.1 | -16.0±2.9 |
| ΔGLS, % | -2.7, p<0.0001 | |
| Negative DSE group (n=24) | | |
| Growth, n=22 (91.7%) | | |
| GLS, % | -18.5±4.0 | -21.6±3.7 |
| ΔGLS, % | 3.1, p=0.011 | |
| Drop, n=2 (8,3%), p<0,0001 | | |
| GLS, % | -20.3±0.3 | -19.5±0.2 |
| ΔGLS, % | -0.8, p=0.09 | |

**Table VI.** CAG results in the studied group

| CA lesions features | Negative DSE group (n=24 (17.1%) | Positive DSE group (n=116 (82.9%) |
|---|---|---|
| 1-vessel disease, n (%) | 2 (8.3%) | 56 (48.3%) |
| 2-vessels disease, n (%) | 0 (0%) | 38 (32.7%) |
| 3- vessels disease, n (%) | 0 (0%) | 22 (19.0%) |
| LCA main lesion, n (%) | 0 (0%) | 8 (6.9%) |
| LAD lesions, n (%) | 1 (4.15%) | 84 (72.4%) |
| Cx lesions, n (%) | 0 (0%) | 38 (32.8%) |
| RCA lesions, n (%) | 1 (4.15%) | 55 (47.4%) |

Combined ΔGLS and ΔWMSI for ischemia diagnosis showed significantly lower sensitivity 86.2% (p=0.0002) and specificity 80.4% (p=0.0064) compared to integral evaluation of ischemia markers, myocardial viability and coronary reserve with significantly lower general method accuracy (AUC 0.83, p<0.0001).

Given lower ΔGLS and ΔWMSI accuracy according to existing publications compared to general DSE with STE test result with myocardial viability and coronary reserve evaluation [3], as well as demonstrated ability of GLS to significantly grow during stress in substantial proportion of pts (32.8%, p=0.0032) with definitely positive test result, in our routine practice we decided to no longer concentrate on GLS or WMSI dynamics. Today we prefer to evaluate GLS in each individual patient, concentrating rather on general test result taking into account myocardial viability and coronary reserve in each coronary territory. Such decision is also confirmed by weak correlation between peak stress GLS and general DSE with STE test result (r = 0.43, p<0.0001) and significantly lower sensitivity and specificity of combined ΔGLS and ΔWMSI quantification with significantly lower method accuracy (AUC 0.83 vs. 0.98, p<0.0001).

According to DSE with STE the majority of positive result pts (114 (98.3%) pts) had viable myocardium, of which 94 (81.0%) had insufficient coronary reserve. The rest (22 (19.0%) pts) had significant coronary lesions with sufficient coronary reserve or well developed collaterals according to CAG. According to DSE and CAG results 96 (82.3%) pts underwent different revascularization procedures: 86 (89.6%) PCI's (54 (56.3%) pts – 1-vessel stenting; 32 (33.3%) pts – 2- vessels stenting). 10 (10.4%) pts with multi-vessel disease underwent CABG with good close results (Table VII).

**Table VII.** DSE with STE and CAG results regarding myocardial revascularization in the studied group

| Results | Total (n = 58) |
|---|---|
| Positive DSE results, n (%) | 116 (82.9%) |
| Viable myocardium, n (%) | 114 (98.3%) |
| Insufficient coronary reserve, n (%) | 94 (81.0%) |
| Sufficient or high coronary reserve, n (%) | 22 (19.0%) |
| Interventions as per DSE results, n (%) | 96 (82.3%) |
| PCI's as per DSE results, n (%) | 86 (89.6%) |
| 1-vessel stenting, n (%) | 54 (56.3%) |
| 2- vessels stenting, n (%) | 32 (33.3%) |
| CABG as per DSE results, n (%) | 10 (10.4%) |

Thus, general result of DSE with STE results evaluation regarding positive or negative test criteria, myocardial viability and coronary reserve evaluation and defining indications for intervention and revascularization (according to CAG – the "golden standard" of verification) showed that DSE with STE has sensitivity 97.9% and specificity 91.7% (AUC 0,95) with identical respective positive and negative predictive value with high relative risk (OR = 564.0, p<0.0001).

It is known that quantitative and semiquantitative 2D-strain assessment allows much better LV and RV regional wall motion abnormalities detection at rest [2,4-6,9,12,14,15]. Also, there is strong evidence that STE is a perfect visualization tool for DSE [2,10,13], especially taking into account the fact that rather often conventional EchoCG parameters in primary pts with CAD suspicion do not really differ.

B-mode strain may be quantified at any pharmacological stress stage during DSE, making it valuable instrument for ischemia diagnosis and myocardial viability and coronary reserve evaluation at rest and stress [2,10,16]. It has been shown that severe ischemia markers, namely longitudinal strain abrupt drop may take place at earliest DSE stages long before visual regional contractility impairment [2,8], which is fully supported by our study results.

Some studies show that longitudinal strain has higher diagnostic accuracy compared to circular and radial strain, and is at least as accurate as visual regional wall motion abnormalities evaluation in case of CAD diagnosis expertise [2]. Taking into account the fact that subendocardial myocardial layer is the most sensitive to ischemia in combination with simplicity and speed of strain quantification during DSE, in our clinical center we chose to use only 2D-longitudinal peak strain as an optimal ischemia diagnosis parameter, which our study results clearly demonstrate.

Our data confirm high safety of DSE in the pts with CAD suspicion, since we saw very rare clinically significant complications requiring additional medication treatment or intervention (2 (1.72%) cases of AF paroxysm cardioversion). Most cases of relatively rare arrhythmia ischemia equivalents were transitory, stopped spontaneously after dobutamine infusion stop and were hemodynamically insignificant. Thus, we consider DSE the safest stress test in the pts with CAD suspicion.

The described cases of severe dynamic LVOT obstruction during DSE in the pts with previously undiagnosed HCM are of particular interest and should be a matter for separate studies.

## LIMITATIONS

We did not limit pts inclusion by body mass index, including obese pts to avoid possible bias and DSE feasibility overestimation due to only optimal visualization analysis. Since the aim of our study was mainly the assessment of myocardial viability and coronary reserve, we did not limit pts inclusion by any LV EF value. Certain limitation might be in technical issues during STE due to suboptimal visualization, especially in high HR cases.

## CLINICAL PERSPECTIVE

Myocardial deformation parameters, namely global and regional peak systolic strain, allow ischemia detection in early stages, which lead STE to take a significant niche in our center routine management of CAD pts requiring better risks stratification. Appropriate software algorithms development in the future might allow more effective implementation of DSE with STE into routine management of pts after ACS.

# CONCLUSIONS

The present study allows considering DSE with semiquantitative 2D-longitudinal STE a safe optimal method for ischemia diagnosis and myocardial viability and coronary reserve evaluation in the pts with CAD suspicion. Given significantly lower ΔGLS and ΔWMSI quantification accuracy along with demonstrated GLS increase ability during DSE in a substantial proportion of pts, authors recommend integral general test interpretation (ischemia markers, myocardial viability and coronary reserve evaluation) rather than concentrating on GLS dynamics by itself.

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ORIGINAL ARTICLE

# EVALUATION OF THE TREATMENT EFFECTIVENESS OF GINGIVITIS BY THE ORAL DYSBIOSIS INDEX IN PATIENTS WITH DIFFERENT REACTIONS OF PSYCHOPHYSIOLOGICAL MALADAPTATION

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## ABSTRACT

**The aim:** The purpose of this study was to evaluate the dynamics of changes of oral cavity dysbiosis index in patients with various reactions of psychophysiological maladaptation after treatment of gingivitis in different observation terms.

**Materials and methods:** Study was conducted on the basis of Department of Therapeutic Dentistry of Ternopil National Medical University during 1 year from January 2018 till January 2019. The study involved treatment of 58 patients 19-44-years old with gingivitis on a background of different psycho-physiological maladaptation reactions, of whom was formed the I main group (37 people) and the II control group (21 people). For evaluation of the effectiveness of the proposed treatment and prevention complexes, we determined the dynamics of changes of oral cavity dysbiosis index after the treatment of gingivitis at different study times.

**Results:** In patients with gingivitis lysozyme activity increased by 15.02% ($p < 0.01$) and urease activity decreased by 24.46% ($p < 0.05$), according to before-treatment data. In patients of the first group, the oral dysbiosis index decreased in 1.5 times fold with respect to before-treatment data ($0.37 \pm 0.09$ vs. $0.56 \pm 0.08$, $p > 0.05$).

**Conclusions:** Therefore, as a result of the use of proposed treatment and prevention complex was able to increase the activity of lysozyme and reduce urease activity, which generally improved the oral cavity dysbiosis in the main group, which after 1 year of observation was equal to the medium degree in patients with all types of psychophysiological reactions.

**KEY WORDS:** gingivitis, psychophysiology, lysozyme, urease, dysbiosis

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## INTRODUCTION

According to WHO, periodontal tissue diseases remain unresolved in modern medicine, and their relevance is determined by the high demand of the population for the effective treatment of these diseases, and most importantly – prevention. Among the risk factors are crucial disorders of oral microbiocenosis and impaired dynamic equilibrium with the body's adaptive immune system, lack of antioxidant protection and transcapillary metabolism in the dental tissues. In the pathogenesis of the inflammatory process of the oral cavity, not only nonspecific protection, but also specific, associated with the function of adaptive immunity plays an important role. It was found that in saliva concentration of sIgA, IgA, IgG, IgM increased, also levels of IL-8, IL-1, but decreased content of IL-4 [1-8].

The role of psychological characteristics of the personality in the emergence and development of dental diseases has been substantiated by a number of studies of domestic and foreign scientists, in which features of the emotional and personal sphere of patients with different resistance to diseases of hard tissues of teeth and periodontium were considered. The analysis of the various factors influence on the occurrence of periodontal tissue diseases indicate that stress is a determining factor in the development of this pathology. In recent years, research on the relationship between human psychological status and periodontal disease has been published and analyzed by scientists at the American Academy of Periodontology. As a result, 57% of studies confirmed a direct relationship between the development of periodontal disease and psychological factors [9-14].

The polyetiology and complex multifactorial pathogenesis of periodontal tissue diseases involves a comprehensive approach to the administration of drugs, depending on the depth of periodontal tissue damage and disease course. Given the dominance of endogenous, local and general factors, first of all, the activity of the microbiological condition of the periodontium and oral cavity; hygiene factors, salivary gland function, functionality of neurohumoral factors, intoxication, lipid peroxidation, manifestations of dystrophic imbalance and others – the clinician needs to determine the benefits of an existing individual complex and appoint a well-founded etiologic and pathological medicines [15-18].

## THE AIM

The purpose of this study was to evaluate the dynamics of changes of dysbiosis index of oral cavity in patients with various reactions of psychophysiological maladaptation after treatment of gingivitis in different observation terms.

## MATERIALS AND METHODS

The clinical stage of the research was conducted on the basis of Department of Therapeutic Dentistry in Ternopil National Medical University during 1 year from January 2018 till January 2019. The study involved treatment of 58 patients 19-44-years old with gingivitis on a background of different psycho-physiological maladaptation reactions, of whom was formed the I main group (37 people) and the II control group (21 people). The following psychophysiological states were considered in the study: without reactions of psychophysiological maladaptation (WRPD), reactions of psychophysiological maladaptation (RPD), somatogenic asthenic symptom complex (SASC), acute neurotic disorders (AND) and neurotic disorders with prolonged course (NDPC).

Further studies were aimed at assessing changes in dysbiosis index of oral cavity after complex treatment of gingivitis at different observation times (3, 6, 12 months). Dysbiosis index (DI) was determined by the formula proposed by prof. Levytskyi A.P. [19] using the average activity results of urease and lysozyme:

$$DI = \frac{\text{Urease activity}}{\text{Lysozyme activity}} \times 100$$

Results interpretation:

- 0,15-0,30 – moderate dysbiosis;
- 0,30-0,50 – average dysbiosis;
- 0,50 and > – strong dysbiosis.

For determination of DI, the activity of lysozyme in the oral fluid was definitited by serial dilutions with the test microbe Micrococcus lysodeikticus. The urease activity was determined by the ability of this enzyme to break down urea with the formation of ammonia, which is quantified using a Nessler reagent. The statistical processing of the results was carried out using standard statistical analysis programs InVivoStat v.3.0, SofaStat v.1.4.6. and Libre Office Calc. v.5.2.2.2.

## RESULTS

In the first group treatment was carried out in accordance with our developed treatment and prevention complexes: hygienic training and education; individual selection of oral care products and professional oral hygiene. Also, local procedures in I group included:

- antiseptic rinsing the mouth with rinses containing chlorhexidine bigluconate ("Perio-AID 0,12%", "Meridol Med-CHX – 0,2%", "Curaprox Curasept 0,12%", "Eludril 0,10%");
- applications of Perio-AID gel on gums, which were carried out by:

- 3 times per day during 4 days for patients with RPD and WRPD;
- 3-5 times per day during 5 days with SASC;
- 5-7 procedures per day, during 7 days with AND and NDPC;

Assignment of drugs of general effect in patients of group I was carried out with the consultative assistance and under the supervision of related doctors of the relevant specialization.

For general strengthening of the body the drug – "Triovit" (KRKA):

- 1 capsule once a day during 1 month for persons with RPD, WRPD, SASC;
- 1 capsule twice a day during 2 months for AND and NDPC.

"Magne-B6 stress control" (Sanofi) containing anhydrous magnesium citrate and pyridoxine hydrochloride to enhance the body's stress resistance. The drug was prescribed: 1 tablet 3 times a day during 1 month for persons with RDP, WRPD, SASC; 2 tablets 2 times a day for 1 month with AND and NDPC;

"Calcemin" (Bayer) was prescribed to improve bone remodeling. The drug was prescribed (after taking "Magne-B6"): 1 tablet a day for 1 month for persons with RPD, WRPD and SASC; 2 tablets a day for 1 month, with a repeated course of 3 months for AND and NDPC.

The treatment scheme of II group patients included: professional oral hygiene, prescribing topical antiseptics, non-steroidal anti-inflammatory drugs depending on the severity of periodontal tissue diseases, sanitation of the oral cavity (according to the order of the Ministry of Health of Ukraine).

As a result of the treatment of gingivitis, the dynamics of changes of DI in different terms of observation was evaluated (tab.1). ***After 6 months***, lysozyme activity increased by 12.51% in I group, ($p < 0.01$) and by 6.08% in II group, ($p<0.05$) in relation to before-treatment data. However, in II group, the activity of lysozyme in the oral fluid was significantly lower than in patients of I group: WRPD – 8.26%, with RPD – 5.93%, ($p1 < 0.05$), SASC – by 2.81%, in the AND – by 4.94% and in the NDPC – by 10.96%, ($p1<0.01$). At this time of the study, the urease activity in oral fluid was significantly decreased in relation to before-treatment data in individuals of I group WRPD – by 29.42% and in RPD – by 27.64%, ($p<0.05$). In other psychophysiological states of patients in the study groups, urease activity though decreased, but the obtained indices did not differ in statistical significance from baseline values ($p>0.05$).

In I group, after 6 months, the oral DI, although decreased, but only in patients WRPD and RPD was treated as moderate with the corresponding values of $0.24 \pm 0.06$ and $0.29 \pm 0.07$, ($p>0.05$). With SASC and AND, the degree of oral dysbiosis was regarded as average, and with NDPC – strong, ($p> 0.05$). In the II group with RPD, WRPD, SASC was determined by the average degree, and in patients with PLR and NDPC – a strong degree of oral dysbiosis ($p>0.05$).

***After 12 months*** of studies in I group was determined a further increasing of lysozyme activity in the oral fluid: WRPD – by 12.94%, with RPD – by 12.10%, with SASC – by 14.28%, in the AND – by 17.09%, and at NDPC – by 20.0%, ($p<0.01$). In II group, lysozyme activity at this study period was equal to the before-treatment data ($p\geq0.05$) and was significantly lower in relation to the values of I group: WRPD – by 10.55%, with RPD – by 9.0%, with SASC – by 10.21%, with AND – by 9.59%, and in NDPC – by 10.96%, ($p1 < 0.01$).

After 12 months of studies was determined a significant decreasing of urease activity in I group, which in individuals WRPD was 30.48%, with RPD – by 28.64%, with SASC – by 24.44%, with AND – by 21.30% and at NDPC – 20.27% lower

**Table I.** Dynamics of lysozyme and urease activity, oral dysbiosis index in groups at different observation times

| Terms | Parameters | Groups | WRPD | RPD | SASC | AND | NDPC |
|---|---|---|---|---|---|---|---|
| Before treatment | *Lysozyme, mcg/ml* | I+II | 487,00±8,62 | 446,00±6,14 | 420,00±5,20 | 392,00±4,56 | 365,00±4,20 |
| | *Urease, umol/l* | I+II | 1,87±0,16 | 1,99±0,15 | 2,25±0,18 | 2,63±0,19 | 2,91±0,18 |
| | *Dysbiosis* | I+II | 0,38±0,09 | 0,45±0,08 | 0,54±0,09 | 0,67±0,10 | 0,78±0,11 |
| After 6 months | *Lysozyme, mcg/ml* | I | 545,00±7,20°° | 489,00±6,13°° | 463,00±5,10°° | 445,00±4,52°° | 428,00±4,18°° |
| | | II | 500,00±7,15°,* | 460,00±6,18°,* | 450,00±5,15°°** | 423,00±4,50°°** | 400,00±4,19°** |
| | *Urease, umol/l* | I | 1,32±0,15° | 1,44±0,15° | 1,75±0,18 | 2,18±0,17 | 2,39±0,17 |
| | | II | 1,60±0,14 | 1,70±0,14 | 1,98±0,15 | 2,30±0,16 | 2,55±0,16 |
| | *Dysbiosis* | I | 0,24±0,06 | 0,29±0,07 | 0,38±0,09 | 0,49±0,12 | 0,56±0,14 |
| | | II | 0,32±0,08 | 0,37±0,09 | 0,44±0,11 | 0,54±0,13 | 0,64±0,18 |
| After 12 months | *Lysozyme, mcg/ml* | I | 550,00±7,15°° | 500,00±6,12°° | 480,00±5,00°° | 459,00±4,52°° | 438,00±4,20°° |
| | | II | 492,00±7,20** | 455,00±6,15** | 431,00±5,18** | 415,00±4,20** | 390,00±4,19** |
| | *Urease, umol/l* | I | 1,30±0,15° | 1,42±0,16° | 1,70±0,17° | 2,07±0,16° | 2,32±0,16° |
| | | II | 1,69±0,14* | 1,80±0,14* | 2,07±0,17* | 2,16±0,16* | 2,41±0,17* |
| | *Dysbiosis* | I | 0,24±0,08 | 0,28±0,09 | 0,35±0,09 | 0,45±0,11 | 0,53±0,12 |
| | | II | 0,34±0,08 | 0,40±0,10 | 0,48±0,12 | 0,52±0,13 | 0,62±0,15 |

Notes:
* p<0,05; ** p<0,01; *** p<0,001– a reliable difference in values for data before treatment
° p<0,05; °° p<0,01; °°° p<0,001. – a reliable difference in values for data between main and control groups.

in relation to before-treatment data (p<0.05). In II group, the values of urease activity were equal to the initial data and were in persons: WRPD – by 30.0%, with RPD – by 28.57%, with SASC – by 21.76%, and in the AND – by 4.34% and for NDPC – 3.87% higher relative to similar values in I group individuals (p<0.05).

The dysbiosis index of the oral cavity of the studied I group in persons WRPD and with RPD was treated as moderate; with SASC and AND, the average and with NDPC was strong (p>0.05). In II group patients with RPD, WRAP, SASC, was determined as average and in the AND, NDPC were strong dysbiosis index.

## DISCUSSION

Periodontal diseases have been and still remain one of the most common and topical dental diseases. Thus, more than 60% of the population over the age of 40 suffer from certain periodontal diseases, this figure increases to 100% in the elderly people. Pathological changes in the periodontium often occur against the background of concomitant diseases – digestive, metabolism disorders, cardiovascular and endocrine diseases, sensitization and infection of the human body [1, 16, 17]. At present time, the relationship between the state of immune protection and the balance of disorders of the prooxidant-antioxidant system have no doubt [20, 21, 22]. The previous statement is explained by the fact that with presence of somatic pathology, typical pathological processes often develop, which are also accompanied by disorders of oxidative metabolism, which weaken the body's functioning of specific and nonspecific protection and lead to pathological and biochemical shifts among components of local immunity in the oral mucosa[5, 6, 23]. All of this become [20, 23, 24]. one of the conditions for enhancing the negative impact of the oral microbiota, which leads to a reciprocal increase in dysmetabolic disorders due to the underlying disease in patient[

At the considering that psychosomatic and psychoneurological diseases can lead to the formation of various defects of the immune protection system, which is a factor in increasing the adverse course of inflammatory diseases in the oral cavity of these patients. Quantitative analysis in the oral fluid of indicators of innate and adaptive immunity allows to personalize the approach to treatment taking into account the severity of disorders of the immune system at the local level. All of the above, makes it possible to perform modern diagnostics and, if necessary, correction of therapy, which increases its effectiveness and reduces the risk of complications in this category of patients[4,18,24,25].

## CONCLUSIONS

One of the important step in treatment of periodontal diseases should be the individual and differentiated development and implementation of local and general therapeutic measures, taking into account the clinical and psychosomatic status of this group of patients. In patients

with gingivitis the lysozyme activity increased, on average, by 15.02% ($p < 0.01$) and the urease activity decreased by 24.46% ($p<0.05$), relative to before-treatment data. The dysbiosis index decreased in 1.5 times relative to pre-treatment data ($0.37 \pm 0.09$ vs. $0.56 \pm 0.08$, $p>0.05$). At the same time, the values of oral dysbiosis index in the second group slightly improved according to the initial data.

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*The Authors declare no conflict of interest.*


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ORIGINAL ARTICLE

# ALEXITHYMIA FORMATION AS AN ADAPTATION TO EVERYDAY STRESS IS DETERMINED BY THE PROPERTIES OF THE NERVOUS SYSTEM

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## ABSTRACT

**The aim** of the study was to determine the psychological nature and mechanisms of alexithymia formation by way of the analysis of its relation to the properties of the nervous system, mental states, and characteristics of the emotional sphere of the personality.

**Materials and methods:** In the process of the study, for the diagnostics of alexithymia, we used the 26-item Toronto Alexithymia Scale (TAS-26) developed by G.J. Taylor and a block of psycho-diagnostic methods aimed at the diagnostics of properties of the nervous systems, the emotional sphere and mental states of respondents. The relationships were evaluated using Spearman's rank correlation coefficient and Pearson's correlation coefficient.

**Results:** The main factors related to alexithymia were weak nervous system, low stress resistance and such characteristics of the emotional sphere as marked extraversion, high level of trait anxiety, neuroticism, indirect verbal aggression, low levels of aggressiveness. The emotional exhaustion and reduction of personal achievements, the Resistance Phase, chronic fatigue and depression were the most pronounced within the alexithymia group. The alexithymic personality type demonstrated less developed spatial anticipation.

**Conclusion:** In accordance with our results, the weakness of the nervous system and high Trait Anxiety facilitate the adaption to stressful situations by avoiding and crowding out negative emotions, lead to the inability of verbal description and expression of emotions. A low level of stress resistance conduces to neurotization, chronic fatigue, and emotional burnout. The predominance of refractory and dysphoric reactions causes a negative vision of the situation and can provoke the development of psychosomatic disorders.

**KEY WORDS:** alexithymia, the emotional sphere, mental states, features of nervous system

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## INTRODUCTION

The problem of alexithymia has been started to be discussed in the scientific psychological literature relatively recently, and the first one to use this term was the French scientist P. Sifnenos in 1973. The term "alexithymia" means the absence of words for description of feelings (a – "absence", lexis – "word", thymos – "emotion") [1].

Alexithymia is manifested in such particularities of the personality as problems in the recognition and verbalization of own emotions and of emotions of surrounding persons, the difficulty in discrimination of emotions and physiological feelings, a "concrete" way of thinking, the decline of the ability to fantasize, more focusing on external events to the detriment of internal experience, and the deficit of inter-personal communication [2; 3; 4; 5; 6].

The etiology of alexithymia remains undetermined. As to reasons of alexithymia, there are two main approaches, which view it either as primary, genetically determined phenomenon [7; 8] caused by the pathology of the brain, or as a secondary phenomenon occurring in result of psychological traumas [9; 6] or trauma of the brain [10]. The development of alexithymia is associated with organic and functional disorders of various brain structures. As the anatomic correlative of alexithymia (more precisely, of one of its symptoms, the Difficulty Describing Feelings) in cases of bulimia nervosa (BN), the denseness of the grey matter of the parietal lobe of the brain, in particular, of the right angular gyrus, is distinguished [11]. The topographical alteration of functional links in cases of alexithymia in resting state reflects the changes in informational and emotions activating processes [12]. In cases of alexithymia, changes are found in inter-regional structural networks (fronto-insular cortex) associated with social effective processes, perception of emotions and empathy [13]. Alexithymia is associated with multiple neural clusters of reward processing, namely, with the reward and the loss prospect phases, the insula, midbrain, and pons, the inferior and middle frontal gyri, and the dorsolateral prefrontal cortex (DLPFC) [14]. The latter is indicative of alexithymia as an acquired characteristic, a reversible phenomenon which is explained as a psychological defense in the form of opposition, avoidance, and negative emotionality [15; 16].

The adaptation approach allows considering alexithymia as one of the consecutive adaptation reactions to anxiety, on

the one hand [17], and as the factor, which preconditions the formation of the somatization – on the other hand [10; 17]. The phenomenon of alexithymia generates the most interest due to its relation to psychosomatic disorders. According to the alexithymia concept of psychosomatic disorders [18; 19; 20], the limitation of apprehension and cognitive processing of emotions, as well as difficulties with their verbalization, facilitate the intensification and prolongation of physiological responses to negative effects of the external environment, which facilitate the creation of conditions for the development of the psychosomatic symptoms.

Different studies demonstrate the link between alexithymia and the coronary heart disease, arterial hypertension, diabetes mellitus, bronchial asthma, gastric and duodenal ulcer, ulcerative colitis, and malignant tumors [21; 22]. Also, links were confirmed between alexithymia and substance-use disorders [14], alcohol dependency [23], panic disorders and phobias [2; 3; 20; 24; 25], depression [8; 20; 26], and autistic spectrum disorders [13]. The link between alexithymia and the emotional burnout is, possibly, very important, because the latter is potentially a phase of development of the depressive disorder [27]. Genetic factors of the link between alexithymia and depression have to be mentioned [8]. Alexithymia can be an independent risk factor of the emotional burnout [28]. This assumption is supported by positive correlation relationships between alexithymia and the emotional exhaustion and depersonalization) and negative correlation relationships with the sense of family support and personal achievement) [26].

The problem of alexithymic personalities is the inability and impossibility to discriminate own emotions and experiences of other persons; they show low expressivity which makes them indifferent, cold and lacking in understanding for persons surrounding them. They are unable to show to their conversation partner their understanding and to express their compassion, and in result inter-personal relations worsen, the adaptation level within a team decreases, and the person him/herself may feel that he/she is different from the others. A low socio-psychological adaptation, in turn, may lead to the experience of loneliness, isolation, inability to master assistance professions (psychologist, physician, social worker) and decreases the level of satisfaction with life and also promotes the development of bad habits. [29, 30].

In contemporary scientific literature, discussions are held in respect of the nature of the phenomenon of alexithymia, and until now one of the most common has been the hypothesis of the alteration in the interaction between brain hemispheres with the insufficient function of the right hemisphere. Without contradicting the justification of putting forward such hypotheses, we would like to mention that the attempt of explaining the nature of alexithymia by solely physiological reasons is clearly insufficient and not only does not exclude, but also does provide for the necessity of a substantial psychological analysis of this phenomenon, that is, the study of the matter of its psychological nature and mechanisms. The knowledge of these mechanisms can help to develop and to give scientific credence to ways of its psychocorrection and psychotherapy.

## THE AIM

The aim of our research was to determine the psychological nature and mechanisms of the alexithymia formation by way of the analysis of its relationships with the properties of the nervous system, mental states, and characteristics of the emotional sphere of the personality.

The goal: to determine the level of alexithymia in respondents; to determine the correlations between alexithymia and properties of the nervous system (the strength of processes of the excitation and the inhibition, mobility of nervous processes); to find correlation relationships between alexithymia and mental states: emotional burnout, chronic fatigue, depression, anxiety; to show the correlation between alexithymia and low stress resistance, neurotization, neurotizm and the expressiveness of psychological stress; to determine the correlation between alexithymia and aggressiveness and anxiety.

The hypothesis of the research was the supposition about the existence of a correlation between alexithymia and properties of the nervous system, which, in turn, determine particularities of the emotional sphere and mental sates of persons with alexithymia traits.

## MATERIALS AND METHODS

### PARTICIPANTS

The objects of survey were 329 healthy volunteers (242 women and 87 men, aged 18 to 26 years old, $M_{age}$ = 18.91, $SD$ = 1.6 years), first-third year students of the Taras Shevchenko National University of Kyiv (Educational and Scientific Centre "Institute of Biology and Medicine" and Faculty of Psychology) and National Aviation University (Institute of the Humanities), Kyiv, Ukraine. The participants were eligible to enroll in the study if they were ≥18 years old and had no clinical manifestations of mental or cognitive impairment. Exclusion criteria were: the use of psychoactive medication, drug or alcohol addiction and psychiatric or neurological complaints. All participants were tested 1-3 months before the exam time (baseline session).

The study was approved by the Bioethics Commission of Educational and Scientific Centre "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv and written informed consent was obtained from each subject in accordance with the World Medical Association (WMA) declaration of Helsinki – ethical principles for the medical research involving human subjects (Helsinki, Finland, June 1964).

### QUESTIONNAIRES

To determine the level of alexithymia we used 26-item Toronto Alexithymia Scale (TAS-26) developed by G. J. Taylor and co-authors in 1985 [31].

Alexithymia is determined by following cognitive-affective psychological features: difficulties in the determination (identification) and description of own feelings; difficulties in the discrimination of feelings and bodily sensations; decrease in the symbolization ability (feeble imagination and poorness of other imagination manifestations); more focus on external events than on internal experiences. In Russian language, the methodology was adapted in Bekhterev Research Institute [32].

In order to measure the severity of emotional burnout in students, we used the 22-item Maslach Burnout Inventory (MBI) and 84-item Boyko's Syndrome of Emotional Burnout Inventory (SEB) [33; 34] adapted for students by Tukaiev and Vasheka [35]. The sub-scales' Cronbach's alpha in the current sample were 0,807 for Emotional Exhaustion, 0,690 for Depersonalization, 0,807 for Personal Accomplishment (MBI), 0,847 for Anxiety Tension, 0,817 for Resistance, 0,759 for and Exhaustion (SEB).

With the purpose of diagnostics of individual topological properties, the emotional sphere and mental states, we used following psychodiagnosis methodologies. The Temperament Diagnostics Test by Jan Strelau and Hans Eysenck's Personality Inventory (EPI) indicates the individual typological characteristics. These methods allowed for determining the strength of excitation and inhibition processes, mobility of nervous *processes*, tranquility of one's nervous system, and the level of extraversion-introversion and neuroticism [32]. The EPI scales' Cronbach's alpha in the current sample were 0,710 for Extraversion and 0,753 for Neuroticism, whereas for Temperament Diagnostics Test scales Cronbach's alpha was 0,866 for Excitative Processes scale, 0,844 for Inhibitory Processes scale, and 0,819 for Mobility of Nervous Processes.

The Taylor Manifest Anxiety Scale (TMAS) measures the anxiety as general personality trait. The State-Trait Anxiety Inventory (STAI) by C. Spielberger (adapted by Y. Hanin) determines the level of anxiety on the basis of self-assessment scale (high, medium, low anxiety). State anxiety arises as a reaction to socio-psychological stressors. Trait anxiety gives an idea of the individual stress susceptibility base on personality traits [32]. The TMAS scales' Cronbach's alpha in the current sample was 0,887, while STAI's Cronbach's

Also, in order to determine the mental state of the interviewees, emotional reactions the following tests were used:

UN scale (Wasserman questionnaire for express diagnostics of neurotization level) was aimed to evaluate the probability of occurrence of neurotic episodes (a diagnostic scale) and social desirability (control scale). Seven degrees of UN scale indicate acute (negative scores) or low (high positive scores over 30 points) neurotization level. [32]. The sub-scales' Cronbach's alpha in the current sample was 0,856.

V.A. Zhmurov differential diagnostics of depression detects mostly depressed mood or melancholic depression, and makes it possible to establish the severity of the doldrums at the moment on a scale from 1 to 132 points: 1 to 9 points – the depression is not formed, 10 to 24 points—the depression is minimal, 25 to 44 points – the mild depression, 45 to 67 points – the moderate depression, 68 to 87 points—the pronounced depression, 88 to 132 points – the major depression. [32]. The scales' Cronbach's alpha in the current sample was 0,950.

V. Boyko diagnostics of emotional response to environmental stimuli allows to determine the dominant type of emotional reaction, transformation method of internal and external influences in a positive, neutral or negative energy states and behaviour acts. The test considers two parameters: the type of emotional reaction (outward euphoric, inward refractory and outward dysphoric types of emotional reaction) and the nature of the stimuli (positive, neutral or ambivalent, negative) [32]. The scales' Cronbach's alpha in the current sample was 0,822 for Euphoria scale, 0,696 for Refractory scale, and 0,764 for Dysphoria scale.

Boston the social stress test "Lifestyle Analysis" determines vulnerability to stressful situations to the extent of the life tension. [32]. The scales' Cronbach's alpha in the current sample was 0,600

Lemur-Tessier-Fillion Psychological Stress Measure (PSM-25) was used to assess current work-related stress levels on 8-point Likert scale ("not at all" to "greatly") from 25 to 200 points [32]. The scales' Cronbach's alpha in the current sample was 0,928.

E.P. Illyn and P.A. Kovalev aggressive behaviour test is designed to detect the predisposition to a certain type of aggressive behavior (the tendency to direct and indirect verbal aggression, the tendency to direct and indirect physical aggression, the level of expansiveness). Integral test indicator (expansiveness) is diagnosed over 20 points. The scales' Cronbach's alpha in the current sample was 0,672 for Direct Verbal Aggression scale, 0,661 for Indirect Verbal Aggression scale, 0,662 for Indirect Physical Aggression scale, and 0,699 for Direct Aggression scale. 20-item Assinger Relationship Aggressiveness Test is intended to identify the stable level of external aggressiveness [32].

In order to identify the presence of chronic fatigue syndrome we used Leonova`s the Degree of Chronic Fatigue Syndrome Test, aimed to determine the pre-clinical stages of chronic fatigue that may result in a number of pathological states (neuroses, asthenic syndrome etc). The technique allows diagnosing following 4 main groups of symptoms: symptoms of physiological discomfort, general feeling unwell and cognitive discomfort, abnormalities in the emotional-affective sphere, the decrease in motivation and changes in the sphere of social communication and the overall index of chronic fatigue [36].

Statistical processing of data was carried out using the methods of mathematical statistics in the Statistical Package for the Social Sciences (SPSS 22). We used the Kolmogorov-Smirnov test to determine the type of data distribution. Pearson product-moment correlation coefficient and Spearman's rank correlation coefficient allowed to measure how strong the relationship between variables. Hierarchical clustering was used to identify relatively homogeneous groups of observations for given characteristics.

**Table I.** Correlation Relationships between Alexithymia and Properties of the Nervous System (J. Strelau methodology), n=329

| | | | | | |
|---|---|---|---|---|---|
| **Alexithymia** | Strength of Excitation Processes | Strength of Inhibition Processes | Mobility of Nervous Processes | Anxiety | Depression |
| | -0.372** | -0.307** | -0.222** | 0.439** | 0.398** |
| **Alexithymia** | Neurotizm | Neurotization | Trait Anxiety | Indirect Verbal Aggression | Aggressiveness |
| | 0.302** | 0.457** | 0.406** | 0.187* | -0.165* |
| **Alexithymia** | Inward refractory activity | Outward dysphoric activity | Outward euphoric activity | Non-Resistance to Stress, The Boston Test | Psychological Stress (PSM-25) |
| | 0.314** | 0.243** | -0.389** | 0.306** | 0.350** |
| **Alexithymia** | Chronic Fatigue State | Emotional Burnout | Exhaustion | Depersonalization | Reduction of Professional Achievements |
| | 0.389** | 0.410** | 0.343** | 0.243** | -0.415** |

* The correlation is significant at the level 0.05 (2-directional).
** The correlation is significant at the level 0.01 (2-directional).

## RESULTS AND DISCUSSION

Using the correlation analysis and with the use of Pearson's and Spearman's criteria, correlation relationships of alexithymia were determined, which had the inverse directionality with such properties of the nervous system as the strength of excitation processes, the strength of inhibition, and the mobility of neural processes, which indicated the predisposition to alexithymia of persons with weak nervous system (Table I).

A direct correlation was determined between alexithymia and characteristics of the emotional sphere of respondents: neuroticism, Trait Anxiety (Spielberger), depression, neurotization, anxiety (Tailor), inward refractory and outward dysphoric reactions to external stimuli, indirect verbal aggression, and the inversed correlation with the aggressiveness (Assinger) and the outward euphoric reaction to stimuli.

High level of neuroticism and anxiety, in our opinion, leads to blocking of emotional experiences, especially in psycho-traumatic situations, and to their repression from the conscious sphere, which is eventually manifested in the inability of their verbal description and expression. Highly anxious personalities constantly suppress their feelings and emotional reactions, and that becomes usual for them, and feelings themselves become less expressed even outside of stress situation.

It is interesting that alexithymia turned out to have an inverse relationship with aggressiveness, which is indicative of the pattern of hiding their emotions, especially negative ones, which is typical for persons with alexithymia. And the direct correlation with indirect verbal aggression points at their tending to express negative emotions by way of talking about unpleasant people or events with their friends, but not by way of a direct conflict.

A direct correlation was determined between alexithymia and mental states, such as the state of chronic fatigue, psychological stress (PSM-25), low level of ability to cope with stress (the Boston Social Stress Test "Lifestyle Analysis"), emotional burnout according to methodologies of V. Boyko and Christina Maslach.

In our opinion, difficulties in the discrimination of feelings and bodily sensations of alexithymic persons may lead to the impossibility to distinguish the chronic fatigue from the state of depression. Herewith, the lack of understanding of own emotions brings up the issue of the adequacy of answers of alexithymic persons to questionnaires, results of which are based on self-reports of respondents. If a person does not understand own feelings, how veracious will be their answers related to them?

The hierarchic cluster analysis was conducted with the use of the Between-Groups Linkage method. In result, the tree diagram shown on Fig. I was obtained, from which it can be understood that alexithymia forms a cluster with properties of the nervous system (the strength of excitation, the strength of inhibition processes, and the mobility of nervous processes). At further steps, this cluster is joined by emotional burnout and psychological stress, which, in turn, join clusters of negative emotions and mental states. In our opinion, obtained results are indicative of the first priority of the nervous system properties in the formation of both manifestations of alexithymia and of the chronic fatigue, exhaustion, stress, etc.

Results of the correlation analysis show the inverse relationship between alexithymia and the strength of the nervous system, that is, average and low strength of excitation, inhibition and mobility of nervous processes is mainly observed in persons with alexithymia. Persons with weak nervous system cannot withstand durable exertion, get tired very soon, are unable to efficiently work for a long time, demonstrate weak self-control and slow reactions to stimuli. In our opinion, properties of the nervous

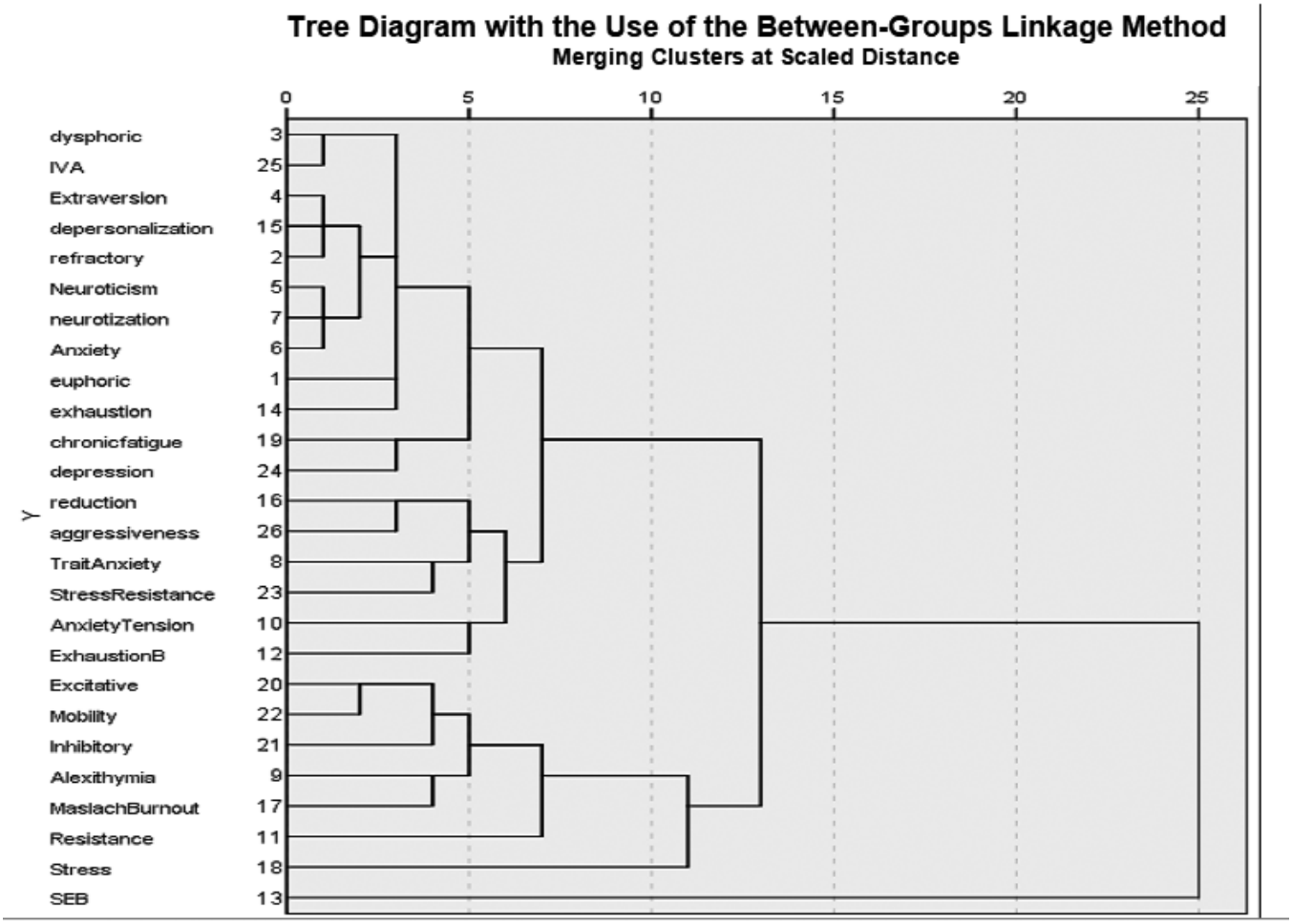


**Fig. I.** Results of Hierarchic Cluster Analysis

system (the low level of the of excitation and suppression processes) determine the low level of resistance, the submission to stress, which, in turn, forms chronic fatigue, neurotization and emotional burnout in persons with alexithymia. Also, a weak nervous system determines the sensitivity to external stimuli, including to pain, which was experimentally determined by Nyklíček et al [37]. Their experiments demonstrate that patients with alexithymia are hyper sensitive to both unpleasant internal somatic sensations and to external pain stimuli.

The emotional sphere of persons with alexithymia demonstrates the vulnerability to external stimuli, which is manifested by emotional instability, predominance of refractory and dysphoric reactions, depressed states, in the high level of Trait Anxiety. On the other hand, in the study by V. G. Ragozinskaya [38] the assumption is made about the psycho-traumatic nature of alexithymia, according to which alexithymic traits develop as a psychological defense mechanism on the basis of previous anxiety and depression. Based only on results obtained during the study, it cannot be definitely asserted what is the reason and what is the cause: alexithymia or high anxiety level. However, the relation between these two psychological characteristics is incontestable.

Also, tight relations are observed between alexithymia and negative emotional states and such properties of the personality as neuroticism and low stress resistance. In our opinion, due to weak nervous system and low resistance to stress, persons with alexithymia are very vulnerable to difficulties and life and psycho-traumatic factors. They react to stress by the development of emotions blocking (at first, of negative ones, and, later, of positive too), which eventually forms into a persistent unawareness of own feelings.

The lack of understanding of own feelings and of feelings of surrounding people worsen the socio-psychological adaptation of alexithymic persons and leads to the sense of loneliness and experiencing own difference, which decreases the level of satisfaction with life and may form depressive disorders.

The dominant type of response to life circumstances are refractory and dysphoric reactions, which points at the predomination of negative apprehension of situations, continuous rueful feelings over an event, fixedness on negative emotions and the strive not to demonstrate such emotions to others. A pronounced anxiety and the inability to react in a positive way lead to high vulnerability to stress influences and to the formation of a specific avoiding style of behavior.

If alexithymia is viewed as a defense from negative psycho-traumatic experiences, the relation between alexithymia and various forms of addictive behavior and overeating becomes understandable [39]: an alexithymic person abstracts from negative emotions by way of searching for new sources of positive emotions, which are not always safe for health.

## CONCLUSIONS

In result of the research conducted, a conclusion can be made that alexithymia as a psychological phenomenon has various natures and manifestations, and due to that no firm conclusion can be made today in respect of its mechanisms. The obtained result of the correlation between alexithymia and properties of the nervous system is indicative of the fact that the predomination of weak nervous system in alexithymic persons, in combination with the high level of neuroticism and Trait Anxiety becomes the trigger mechanism for blocking negative emotions, which, in the course of time, may develop into a generalized process of suppression of entire emotional experiences.

The relations between alexithymia and such mental states as the emotional burnout, chronic fatigue and depression which were determined allow supposing that, firstly, described phenomena are similar in a way, which requires a long-term longitudinal study of the dynamics of mental states, and also confirms the assumption in respect of the vulnerability of alexithymic personalities to the influence of negative stimuli and difficult life situations. The predomination of refractory and dysphoric reactions to stimuli complicates the positive rethinking of a situation by alexithymic persons and worsens their emotional state. Also, in our opinion, refractory reactions directed inwards (accumulation of resentment or anger) in combination with indirect verbal aggression and low level of aggression lead to negative experiences and is an impulse for the development of psychosomatic disorders.

In prospect, of special interest are the studies of specific features of the cognitive sphere of alexithymic personalities and of their ability of anticipation and creativity, which may become the subject matter of future studies in this field.

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**Conflict of interest:**

*The Authors declare no conflict of interest*

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ORIGINAL ARTICLE

# PATHOPHYSIOLOGICAL AND PATHOMORPHOLOGICAL ASPECTS OF RELAPSE OF VARICOSE VEINS AFTER ENDOVASCULAR LASER VEIN COAGULATION

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## ABSTRACT

**The aim:** With the help of biochemical and morphological methods of investigation to identify the causes of a false postoperative recurrence of varicose veins after the EVLC.

**Materials and methods:** In 173 patients with varicose veins of the lower extremities, the level of markers of endothelial dysfunction was determined: P-selectin, E-selectin, tissue plasminogen activator, endothelin-1, adhesion molecules of type 1 vascular endothelium (sVCAM-1-soluble vascularcellularmolecula), circulating endothelial cells (CEC) before surgery (EVLC), on the 10th and 60th day of the postoperative period.At the same time, a morphological and electron microscopic examination of the state of the deep venous system in 31 patients with varicose vein disease of the lower extremities who died from acute heart failure, was performed.

**Results:** Increased values of markers of endothelial dysfunction in patients with varicose veins of the lower extremitiesbefore surgery of EVLC were established. We found that, despite the operation, the parameters of endothelial dysfunction decrease, but in the remote postoperative period do not come to the norm. Morphological and electron microscopic studies of the deep vein wall revealed pathomorphological changes in all of their layers, especially the endothelial layer. At the heart of the development of endothelial dysfunction in the postoperative period, the leading role belongs to changes in mitochondria.

**Conclusions:** 1. Based on our research, we can state that there are significant pathomorphological and pathophysiological changes in the deep venous system of the lower extremities in conditions of varicose vein disease.

2. The initiator of postoperative relapse of varicose veins are structural changes in the wall of deep veins with a violation of the integrity of the endothelial lining, contributing to the absorption of plasma and leukocyte contents from the blood stream in the interstitium, with the following pathological changes in the layers of deep veins.Such changes are the basis for the manifestations of endothelial dysfunction in the postoperative period.

**KEY WORDS:** EVLC, varicose veinsrecurrence, endothelial dysfunction

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## INTRODUCTION

Endovascular laser coagulation (EVLC) of veins due to its low traumatic and cosmetic effect became a priority area in the treatment of varicose veins of the lower extremities (VVLE) [1, 2, 3]. Ultrasound diagnostics of the state of the superficial and deep venous system plays a significant role in improvementof functional results after EVLC, which makes it possible to assess the presence of blood flow in the veins, the diameters and their lumen, deformation and saccular transformation of the veins, wall thickness, homogeneity, elasticity of the valves, their function during loading hydrostatic samples, presence of blood reflux, the duration of the retrograde flow along the venous lines, as well as its distribution to the anatomical segments, the state of the sapheno-femoral and sapheno-popliteal junction, to determine the localization and perforator veins, their diameter and duration of venous blood reflux in them [4, 5, 6]. Thus, individual hemodynamic features of the venous system of the lower extremities in a patient are detected and, based on this, the EVLC is selected in combination with other minimally invasive operations.

Thus, individual hemodynamic features of the venous system of the lower extremities in the patient are detected and, based on this, the EVLC is selected in combination with other minimally invasive surgeries. It should be noted that the introduction of highly informative diagnostic technologies and innovative methods of surgical treatment of VVLE significantly reduced the number of postoperative recurrence of varicose veins of lower extremities. At the same time, in the vast majority of scientific publications, the main emphasis of causes of postoperative recurrence of varicose veins of lower extremities is on tactical and technical errors during saphenectomy [7].Thus, according to the analysis of the results of surgiries performed in specialized clinics where the diagnostic, tactical and technical principles of VVLE treatment are observed, the percentage of postoperative recurrence of varicose veins of lower extremities is 5–12.5% [8, 9, 10, 11, 12, 13, 14]. In our opinion, a certain number of postoperative recurrence of varicose veins of lower extremitiesfalls on a “false relapse”, which may be due to the progression of varicose veins, but already the system of deep veins. We did not find a definite answer to this problem in an accessible scientific literature.

## THE AIM

With the help of biochemical and morphological methods of investigation, to identify the causes of a false postoperative recurrence of varicose veins after the EVLC.

## MATERIALS AND METHODS

The results are based on an analysis of data from a study of 173 patients operated on varicose vein disease of the lower extremities.According to the international classification of CEAP, there were 84 patients with $C_2$, 66 – with $C_3$ and 23 patients – with $C_4$. The age of the patients was (45±5.7) years.Therewere 98 women and 75 men.

The functional state of the deep and superficial venous system of the lower extremities was determined by ultrasonic color duplex scanning of veins.To do this, the Vivid 3 (General Electric, USA) device was used with a 5–10 MHz frequency sensor and a corresponding standard software package of the mentioned company to test the venous system.During the ultrasound examination, the presence of blood flow in the veins, the diameters and forms of the lumen of the veins, their deformation and sacrificial transformation, wall thickness, homogeneity, elasticity of the valves, their function during loading hydrostatic tests, presence of blood reflux, duration of retrograde flow through the venous lines, and also its distribution on anatomical segments, a state of sapheno-femoral and sapheno-popliteal junctions. In all patients, the failure of the sapheno-femoral junction with varying length of reflux on the trunk of the large subcutaneous vein was detected.

Endovascular laser coagulation of veins (EVLC) was carried out by the Ukrainian portable high-intensity semiconductor (diode) laser apparatus of "Lika Surgeon", manufactured by Small Production Company "Photoni-Ca Plus" (Cherkasy city) with a wavelength of 1470 nm, with a power of 10–12.5 W.The light guide's position was controlled by laser red pilot radiation, or by inoperative ultrasound diagnostics. Surgical intervention wasperformed under general anesthesia or spinal anesthesia.Crossectomy;endovascular laser coagulation of the stems of the large (small) subcutaneous vein and perforated veins;surgical treatment of tributaries of large and small subcutaneous veins with the use of mini accesses according to Muller and their catheter sclerotherapy were performed;

Endothelial dysfunction was evaluated by determining the level of endothelial dysfunction markers: P-selectin, E-selectin, tissue plasminogen activator, endothelin-1, adhesion molecules of type 1 vascular endothelium(sV-CAM-1-soluble vascular cellular molecula), circulating endothelial cells (CEC).These parameters were studied in all 173 patients.Blood collection was performed intraoperatively by puncture of the ulnar vein of the forearm and stem of the varicose large subcutaneous vein, departing from the sapheno-femoral junction, distal to 2 cm.Concentrations of P-selectin, E-selectin, tissue plasminogen activator, adhesion molecules of type 1 vascular endothelium(sVCAM-1-soluble vascular cellular molecula) were determined using the BenderMedSystems (Austria) set for enzyme immunoassay(EIA) according to the manufacturer's instructions.Concentration of endothelin-1 was established using Biomedica (Canada) sets for EIA according to manufacturer's instructions.The reaction was measured on a SUNRISE (Tecan, Austria) microplate semiautomatic photometer using a Hydroflex washing station (Tecan, Austria), which allowed standardizing these methods.The control was the study of 30 healthy people. Endothelial dysfunction markers were also detected in the early (10 days after surgery) and the late postoperative period (60 days after surgery).In these cases, the blood for the study was taken from the femur of the limb, operated on the VVLE, by puncture.

To determine the CEC, we used the method of J. Hladovec and N.N. Petryshchev and co-athors (2001). Blood collection was carried out in the morning, on an empty stomach, by the puncture of the ulnar vein of the forearm. The blood was taken in a 5 ml test glass S-Monovette (Germany). The method is based on the isolation of endothelial cells with platelets, followed by platelets sedimentation with adenosine diphosphate (ADP). To obtain plasma-rich platelets, immediately after the blood was centrifuged for 10 minutes at a speed of 1000 rpm, then 1 ml of plasma was mixed with 0.23 ml of sodium adenosine diphosphate salt at a concentration of 1 mg/ml.The obtained mixture was mechanically stirred on a shaker ELMI-S3 for 10 minutes, at a speed of 100 rpm. Free platelet supernatant was transferred to another container and centrifuged at 9000 rpm for 10 minutesfor the sedimentation of endothelial cells.Then, the free plasma was carefully removed, and the obtained precipitate was suspended in 0.1 ml of 0.9% sodium chloride solution and mixed with a disposable tip.Hemocytometerwas filled with a finished suspension.The number of cells in the endothelium was counted in 2 chambers by phase-contrast microscopy (Biomed 5, Russia) and divided by 2, to obtain the average result in two chambers.Taking into account the relationship between the number of cells in the grid and the volume of thehemocytometer, the volume of the obtained suspension and volume of plasma, when counting the number of endothelial cells, was multiplied by $10^4$/l. With normal endothelial function, the number of CEC is in the range of 0–4 $10^4$/l.

The regions of the femoral vein were removed, up to 2 cm in lengthfor determination of morphological state of the deep venous system of the lower extremities, in a sectional study, in 31 patients with varicose veins of the lower extremities. Histologic preparations were made according to generally accepted methods. Coloring was done by hematoxylin and eosin. Morphometric studies were performed using a histological analysis system. The image on the computer monitor was output from the LOMO Biolam microscope with a help of Vision CCD Camera and InterVideoWin DVR.

For electron microscopic studies, the venous wall region was pre-fixed in 2.5% glutaraldehyde solution with an active medium pH reaction of 7.2–7.4 prepared on Millonig's phosphate buffer. Post-fixation of vein parts was carried out

**Table 1.** Biochemical markers of ED before surgery in blood samples from a large subcutaneous vein (LSV) and ulnar veins in patients with VVLE (n = 173).

| Index | Control (n=30) | LSV | Ulnar vein |
|---|---|---|---|
| CEC, $\times 10^4$/l | 4.3±1.2* | 7.67±2.3* | 4.6±1.1* |
| sVCAM-1,ng/ml | 234.2±57.6* | 384.2±66.3* | 339.1±58.7* |
| P-selectin, ng/ml | 161.9±22.7* | 201.7±29.4* | 176.7±22.5* |
| E- selectin,ng/ml | 39.6±9.6* | 47.9±11.2* | 43.7±8.6* |
| t-PA, ng/ml | 3.4±1.07 | 2.7±0.6 | 2.0±0.9 |
| Endothelin-1, fmol/ml | 2.2±0.4 | 2.0±1.2 | 1.3±0.1 |

* – $p<0.05$

**Table II.** Comparative characteristics of ED indices before surgery and in postoperative period (n=173)

| Index | Control (n=30) | Before surgery | 10 days after surgery | 60 days after surgery |
|---|---|---|---|---|
| CEC $\times 10^4$/l | 4.3±1.2* | 7,6±2,3* | 9,8±4,3* | 8.02±1.8* |
| VCAM-1, ng/ml | 234.2±57.6* | 384.2±66.3* | 420.9±74.2* | 409.6±83.8* |
| P-selectin, ng/ml | 161.9±22.7* | 201.7±29.4* | 199.6±19.9* | 178.9±32.3* |
| E-selectin, ng/ml | 39.6±9.6* | 47.9±11.2* | 43.8±3.6* | 40.5±4.2* |
| t-PA, ng/ml | 3.4±1.07 | 3.7±0.6 | 3.6±1.04 | 3.9±1.7 |
| Endothelin-1, fmol/ml | 2.2±0.4 | 2.0±1.2 | 1.9±0.9 | 1.5±1.1 |

*- $p<0.05$

with a 1% solution of osmium tetroxide on Millonig's buffer during 60 minutes, after which dehydration of the material in alcohols and acetone was carried out and poured into epoxy resins according to the generally accepted method. Ultrathin vein cuts made on ultramicrotome UMPT-7, stained with 1% aqueous uranyl acetate solution, contaminated with lead citrate according to the Reynolds method, and studied on electron microscope PEM-125K.

Statistical processing of digital data was carried out by the method of variation statistics.The reliability of the difference between mean values and their errors was estimated according to the Student-Fisher test.The processing of digital data was carried out using the Student method in the Exel program on a personal computer.True probability of error was considered to be less than 5% ($p \leq 0.05$).For each investigated value, the arithmeticweighted mean (M) was calculated by the formula: $M = (\Sigma V \times P) / n$, where P – the number of cases of observation of this feature;V – option.

## RESULTS AND DISCUSSION

The analysis of biochemical markers (Table 1) indicates the presence of obvious signs of endothelial dysfunction (ED) in patients with VVLE.

Thus, the number of circulating endothelial cells in the venous blood affected by varicose veins of lower limbswas significantly higher compared with venous blood taken from the ulnar vein.This indicates the location of pathological processes when VVLE and proves the high significance of this marker of endothelial dysfunction in this pathology.

Also, the difference in sVCAM-1 measured in venous blood taken from LSV and ulnar veins was found out.This indicates a violation of the interaction of endothelial cells with peripheral blood cells.Thus, the increased expression of the adhesion molecule sVCAM-1 in the vein wall is a sign of migration of leukocytes through the endothelium and maintaining the inflammatory process in it.The foregoing proves that in the development of varicose disease of the lower extremities, one of the key roles is inflammation against the background of a functionally altered and activated endothelium.

In a repeated study of ED markers in the early (10 days) and late (60 days) postoperative period, we stated that the main indicators of ED were still increased, although at their level they were less than preoperative.Thus, it can be argued, that after saphenectomy, ED is not completely eliminated.

To establish the causes of this phenomenon, we conducted an additional morphological and electron microscopic examination of the state of the deep venous system of the lower extremities in 31 patients with varicose veins of the lower extremities.Material for research (femoral vein regions, up to 2 cm in length) was collected during sectional study.

As a result of investigations of the morphological state of the deep venous system in patients with varicose disease, the following types of morphological changes in their walls were distinguished: a) hypertrophy of structural elements of the wall; b) initial phenomena of venous wall sclerosis on the background of hypertrophy; c) initial phenomena of atrophy of the vein wall on the backgroundof evident sclerosis.

In the first category of patients, changes in the inner membrane were associated with the endothelial layer and the development of arterialization of the venous wall, which was manifested in the appearance of spindle-shaped regionsprotruding in the lumen of the vessel by endothelial cells. The nuclei of the endothelial cells looked bright.There were also small areas ofendothelium desquamation, the formation

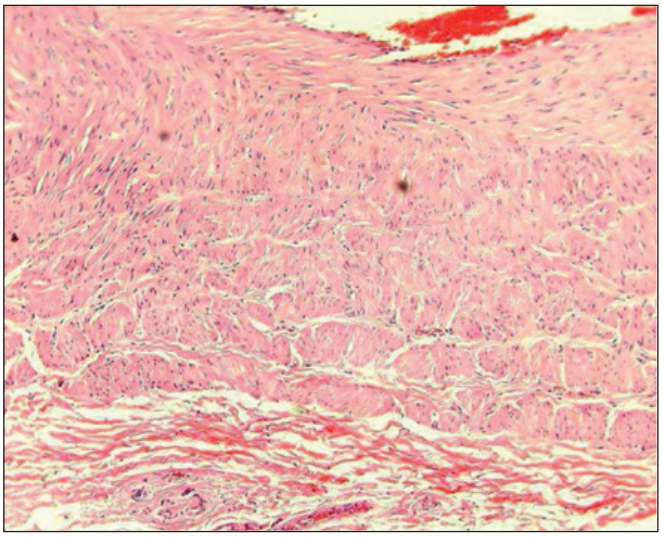

**Fig. 1.** Hypertrophy of the vein wall. Hematoxylin-eosin. Amplification x 100.

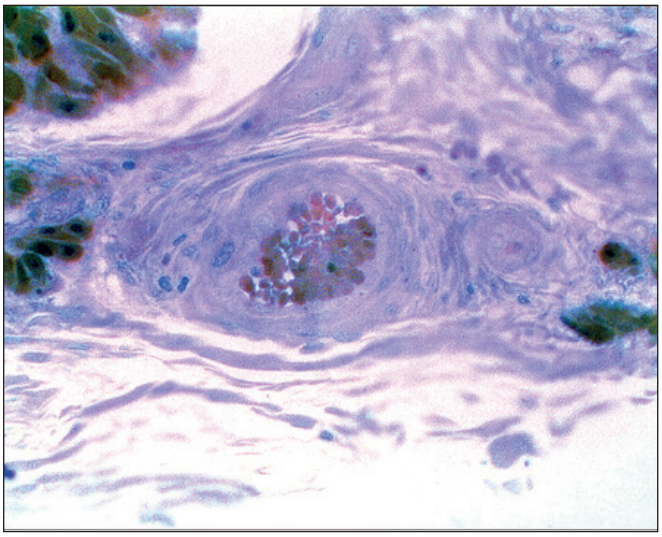

**Fig. 2.** Hypertrophy of the vein wall. The vessel in the vessel. Ultra-thin slice. Amplification × 400

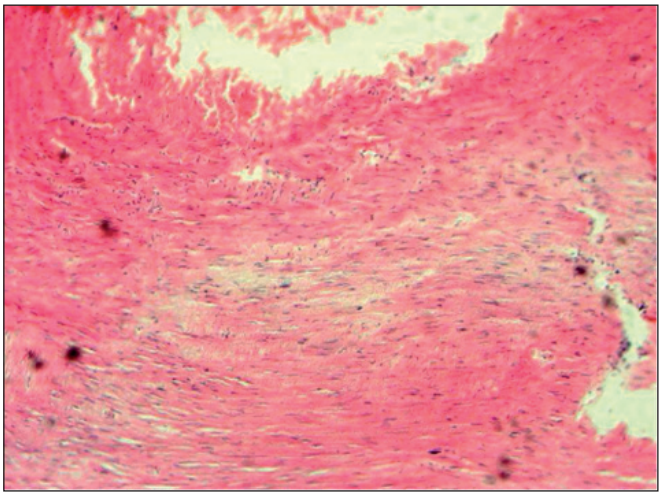

**Fig. 3.** Endothelial hypertrophy, desquamation, development of collagen fibers, collagenosis, fragmentation of individual collagen fibers. Hematoxylin-eosin.Amplification x 100

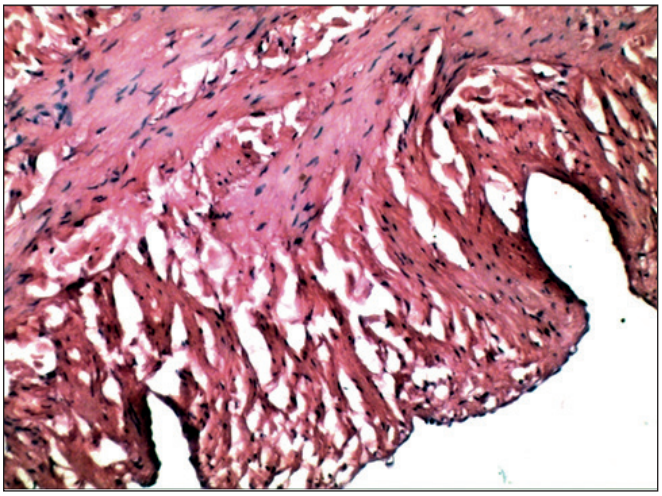

**Fig. 4.** Initial phenomena of atrophy against the background of severe sclerosis.Endothelial hypertrophy, desquamation, development of collagen fibers, collagenosis, fragmentation of individual collagen fibers. Hematoxylin-eosin.Amplification × 100.

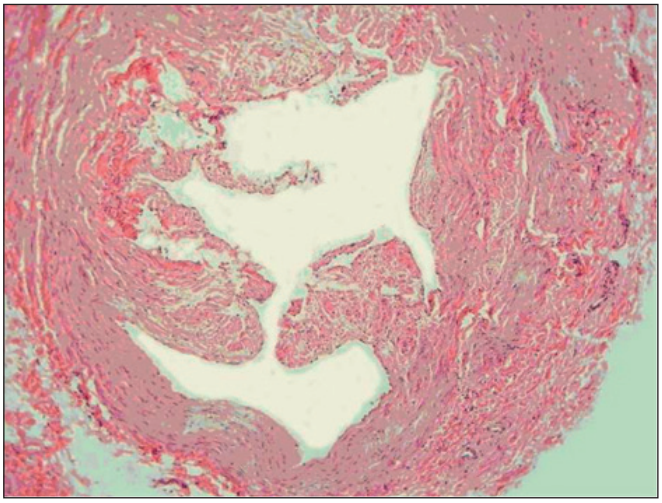

**Fig. 5**. Initial atrophy of the venous system against the background of severe sclerosis.Hematoxylin-eosin.Amplification x 100.

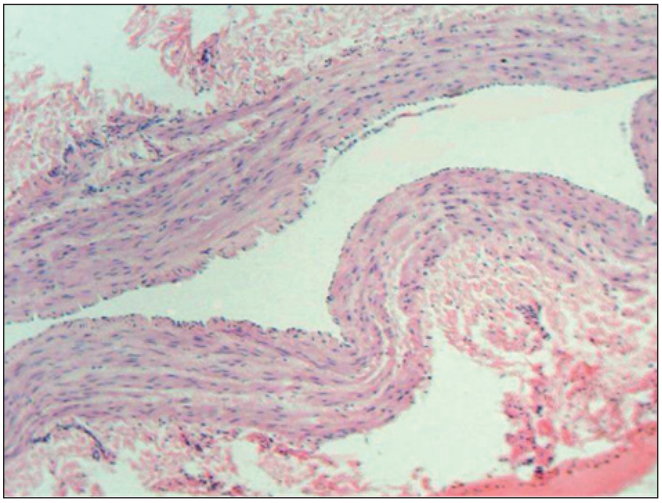

**Fig.6**. Significant atrophy of the venous system against the background of severe sclerosis.Hematoxylin-eosin.Amplification x 100.

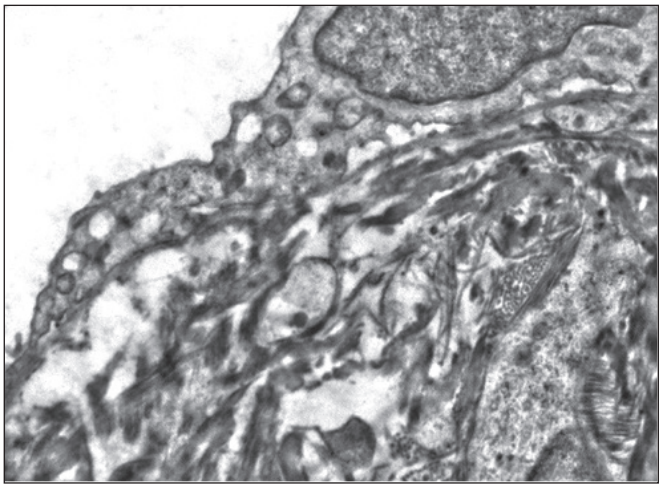

**Fig.7.** Destruction and homogenization of mitochondria, fragmentation of the endoplasmic complex membranes, increase of the number of ribosomes and polysomes.Amplification × 18000.

of multi-nucleated cells of the symplastic structure and the area of hypertrophy of the subendothelial layer.In this case, the subendothelial layer is moderately developed, whereas the muscular layer is constructed of two well-differentiated layers of muscle fibers forming the inner circular and outer longitudinal layer, the fibers are hypertrophied, interfascicular sclerosis is noted among them. The adventitious membrane is thin, sometimes not visualized. The outer and inner elastic membranes are visualized separately. In the upper third of the large subcutaneous vein, in the abdominal region, these elements were more pronounced, and there is also an additional middle layer of the muscular membrane with skewed direction of fibers (Fig. 1).

In the subendothelial layer there is a slight increase in the content of glycosaminoglycans.The valves of the veins were characterized by the presence of a well-developed elastic component of the connective tissue in them.In some cases, the areas of two-sided epithelization were visualized in the valves.

In the second group of patients, we established the changes that characterize the intensive development of collagen fibers, the appearance of a network of collagen fibers on the border between the internal endothelial and middle – muscular layer, in addition, the direction of collagen fibers was parallel to the endothelium, and in some cases at an angle tohim.Elastic fibers in the muscle layer were largely unchanged, occasionally their fragmentation occured.The content of sour glycosaminoglycans was within the normal range.They were also in the muscle of the vessels (Fig. 2), which had a typical structure, in some cases, we noted hyalinosis.In adventitia, elliptical bundles of smooth myocytes, well-developed elastic membranes in the arteries of the vessel were visualized, as well as the usual thickness and number of collagen fibers in the absence of sclerotic changes and, within the norm, the content of acidic glycosaminoglycans.Collagen fibers are thick enough, mostly intact.

In this case, the construction of the endothelial layer is practically no different from the first group.There is a thickening of the subendothelial layer, in some cases, uniform around the vessel, a cluster in the form of a pillow of smooth myocytes with spindle-shaped nuclei, located along the axis of the vessel.Areas of desquamation of the endothelium in places of its hypertrophy (Fig 3–4).Accumulation of acidic glycosaminoglycans, initial sclerosis due to the development of collagen fibers was noted in the subendothelial layer.Elastic fibers on the border with the muscular membrane are thickened, in some cases, even with the phenomena of initial hyalinosis. The muscular membrane contains smooth myocytes with areas of their folding, most pronounced in the outer layers of the membrane. Sclerotic changes in the muscle and their degree were expressed in different parts of the venous wall. On some regions, there was a folding of the muscular membrane with separation of their connecting tissue. The elastic fibers were hypertrophied. Sclerosis with thickening and homogenization of elastic fibers was visualized in the adventitious membrane.

The third group of patients was characterized by the predominance of processes of atrophy of elements of the vascular wall against the background of multiple sclerosis (Fig. 5).

The thickness of the vascular wall was different and characterized by the presence of regions in the form of spindle-shapedknots, there were sites of desquamation of the endothelium. In some regions the spindle-shapedknots of the subendothelial layer, to which smooth myocytes with rounded nuclei are adjacent, penetrated the endothelium into the lumen of the vessels. The subendothelial layer contains a large amount of sour glycosaminoglycans. Fibrinoid

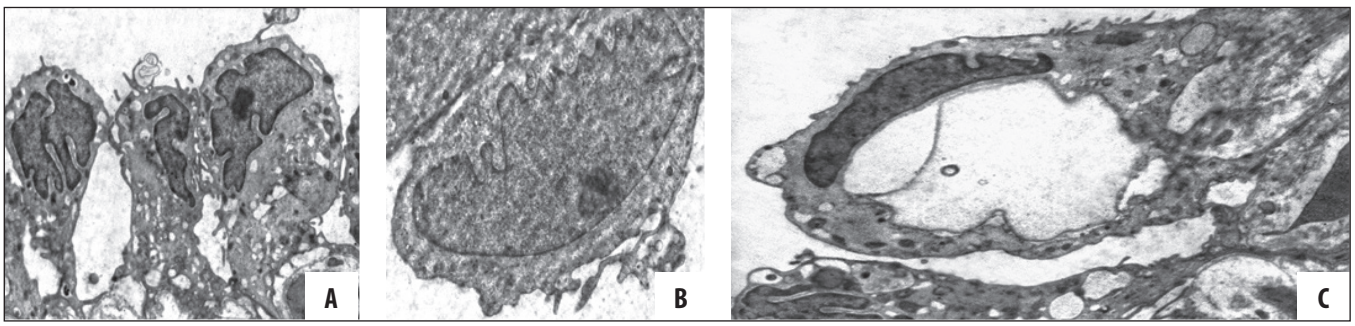


**Fig.8.** (A, B, C).Ultrastructure of endothelial cells of the lower extremities veins with varicose disease.Diluted nuclear membrane, condensed chromatin, expanded perinuclear space, A × 5000, B × 8000, C × 8000.

**Fig. 9.** Ultrastructure of smooth myocytes. Destruction of cytoplasmic organelles × 15 000

and mucoid edema of the connective tissue of the inner membrane of the vein with the phenomena of metachromasia and homogenization of collagen fibers, swelling and folding of elastic fibers of the inner elastic membrane were observed.The muscular membrane was characterized by pronounced atrophic changes of loss of myocytes, elastosis and folding. In some cases, the muscular membrane was represented by separate islets of smooth myocytes lying between thick and coarse collagen fibers, in a raw – absent completely, in a raw– thinned to 2-3 layers of myocytes. Myocytes contained hyperbazophilic, often spiral-twisted, very thin nuclei. There were areas without myocytes in areas of extremely thinned wall in the musclular layer. The adventitious membrane was characterized by pronounced sclerotic changes, elastosis, hyalinosis. In some cases, atrophic changes in the venous wall were characteristic in the same group of patients (Fig. 6).

In the intima there was a complete destruction of the elastic elements with the accumulation of a large number of acidic glycosaminoglycans in the subendothelial layer. In the large part of the vascular wall there was a significant thinning of the muscular membrane with the effects of elastosis and an increase in the number of collagen fibers. In some cases, myocytes were absent, and those that survived acquired structures with a sharp thinning, some elongation, spiral twisting or deformation of nuclei;in the adventitia – a certain thickening of elastic and collagen fibers.

In the electron microscopy study, endothelial cells did not differ significantly from the known cellular indexes of this type and had a standard set of organelles.The nuclei are in most cases with an uneven surface, condensed on the marginal type of chromatin.Micropinocytotic vesicles, which are mainly located near the basal cell surface, testify to normal transendothelial transport in vessels.Endothelial cell mitochondria had an average electron density and a homogeneous matrix.

Cristas of a large part of mitochondria were not reduced, individual mitochondria contained totalized cristas and a bright matrix.The outer membranes were with the islets of destruction (Fig. 7).Ductuli of granular ofendoplasmic reticulum were significantly expanded, and some were electron-transparent vacuoles.There are practically no ribosomes on their membranes, and in the cytoplasm a large number of ribosomes and polysomeswere detected. The gialoplasma of the endothelial cells was moderately enlightened, the centers of lysis of membranes of the granuloid endoplasmic reticulum.Also, in the endothelial layer there were some enlarged cells with an enlightened cytoplasm, which we regarded as edema of endothelial cells.In some endothelial cells, vacuolation of the cytoplasm was determined.In the cytoplasm of the processes there was a large number of micropinocytosis vesicles filled with an electron-transparent substance (Fig. 8).

The cytoplasm of smooth myocytes was filled along fibers with actin and myosin microfilaments, and their cytoplasmic membrane was clear with small lysis foci. Organelles were not always localized in the perinuclear region of smooth myocytes, occasionally placed in the form of aggregates in other parts of the cytoplasm and were surrounded by bundles of filaments.The mitochondria and membranes of the Golgi cytoplasmic complex were destructively altered.

In the wall of the veins in the places of the damaged endothelial layer, there was a contact of erythrocytes directly with myocytes.In these places, red blood cells closed with eachother and underwent focal lysis.

In the places of endothelial layer damage, intracellular processes in fibroblasts intensified.The nuclei of fibroblasts acquired a festoniclook with multiple deep and superficial invaginations of the nuclear membrane.The nuclear chromatin was partially condensed and concentrated near the nuclear membrane.In the central part of the karyoplasm, ribosome clusters are present.Perinuclear spaces were not expanded.

Cytoplasm of smooth myocytes was filled along the fibers with actin and myosin microfilaments, their cytoplasmic membrane is clear with small foci of lysis (Fig. 9).Organelles were not always localized in the perinuclear region of smooth myocytes, occasionally placed in the form of aggregates in other parts of the cytoplasm and were surrounded by bundles of filaments.The mitochondria and membranes of the Golgi cytoplasmic complex were destructively altered.

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In the cytoplasm of fibroblasts, there were well-developed tubules of the granular endoplasmic complex, their tanks

were thinned.On the membranes there are numerous ribosomes.A small number of mitochondria contained cristas, the number of which corresponded to this type of cells, the basal membrane was thinner, had a diverse density.

Numerous vacuoles are concentrated around nuclei, or occupy a considerable area of their cytoplasm. Expressive vacuolation of myeloid cells, as a rule, was accompanied by damage to the myofibrils of these cells, which is an evidence of partial violation of their contractile function. In vasavasorum, there are places where red blood cells accumulate, which completely block the lumen of arterioles, venules and capillaries. Some of them were in a state of hemolysis, which, under ultra microscopy, was shown to be heterogeneous in content density. The tight contact of erythrocytes with endothelial cells testified to the slowing of blood flow. The conducted ultrastructural studies of the organization of endothelial cells in varicose veins revealed a violation of the metabolic activity of organelles.Mitochondria were subject to significant destructive changes, indicating a violation of bioenergy supply of synthetic processes.As a result of the violation of bioenergetic processes, the synthesis of substances decreases as well, manifested by the sharp expansion of the tanks of the granular endoplasmic complex, the disappearance of ribosomes, and the reduction of the Golgi cytoplasmic complex.There were also catabolic processes, as indicated by the increase in the number of lysosomes located near the Golgi complex.

The data of electron microscopic studies made it possible to reveal at the ultrastructural level pathological changes that occur in the layers of the venous wall and draw some conclusions about the pathogenesis of relapse of varicose disease.

So, in our opinion, the basis of the development of endothelial dysfunction and development of varicose disease, the leading role belongs to changes in mitochondria. Mitochondrial insufficiency, manifested by lysis of cristas and external membranes, disrupts bioenergetic processes in cells, while the reparative possibilities of intracellular membrane structures and the metabolism of endothelial cells in general are also reduced. The absence in the cytoplasm of the processes of endothelial cells, micropinocytosis follicles indicates a decrease in the activity of transcellular transport of substances and electrolytes through the endothelium.

Further destructive changes of leiomyocytes indicate a violation of contractile properties of the venous wall and a decrease in their tone.Condensation of nuclear chromatin indicates a decrease in metabolic activity, and destruction of external membranes of mitochondria, disorganization and lysis of cristas and sealing of their matrix is a structural confirmation of the low level of contractile capacity of leiomyocytes under conditions of bioenergy deficiency.And the state of ultrastructural organization of smooth muscle cells in the case of varicose veins of the lower extremities does not allow maintaining a normal vascular tone.Activation of the fibroblast of the venous wall producing collagen and elastic fibers testifies to the compensatory reaction aimed at maintaining the normal configuration of the vessel wall in the places of damage of the endothelial layer.

## CONCLUSIONS

Based on our research, we can state that there are significant pathomorphological and pathophysiological changes in the deep venous system of the lower extremities at VVLE. The initiator of postoperative recurrence of varicose disease is the structural changes in the wall of deep veins with violation of the integrity of the endothelial insulin, which contributes to the contents of plasma and leukocytes from the bloodstream in the interstitium, with the following pathological changes in the layers of deep veins. Such changes are the basis for the manifestation of endothelial dysfunction in the postoperative period. There is a need to develop criteria for predicting postoperative recurrence of varicose veins, as well as means to prevent its development.

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ORIGINAL ARTICLE

# ROLE OF PROINFLAMMATORY CYTOKINES IN PATHOGENESIS OF ARTHROPATHIES IN PATIENTS WITH DIABETES MELLITUS

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## ABSTRACT

**The aim:** Of our work was to study the level of proinflammatory cytokines in patients with diabetic arthropathy and to investigate their possible effect on the development of this complication.

**Materials and methods:** 118 patients were examined, which were divided into groups by type of diabetes, the presence and severity of diabetic arthropathy. The content of IL-1, TNF-α, IL-6 and receptors to S IL-6-R in serum was determined by immunoassay.

**Results:** In patients with diabetic arthropathy, levels of TNF-α (with type 1 diabetes 44.5%, type 2 diabetes 42.9%) and IL-6 (with type 1 diabetes 52.1%, with diabetes 2 types by 64.4%) significantly increased. There is a direct correlation between the severity of joint damage and the level of TNF-α and IL-6. For IL -1, receptors for S IL-6-R have not been detected.

**Conclusions**: The chances of detecting arthropathy with type 1 diabetes with increasing TNF levels increase by 1.7 times, with an increase in IL-6 by 1.5 times. For type 2 diabetes, it is 1.8 and 1.3 times, respectively. Thus, TNF-α and IL-6 may be markers of the presence and progression of arthropathy in patients with diabetes mellitus

**KEY WORDS:** diabetes mellitus, diabetic arthropathy, joints, chondrocytes, cytokines

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## INTRODUCTION

Diabetes (DM) is still one of the most important medical and social problems of the present since diabetes-related morbidity and disability rates are continuously increasing and incidence of its severe and complicated forms is also growing with every coming year. To date, in Ukraine there have been registered more than 1 million patients with DM. However, experts believe that this reflects just the tip of the iceberg; the real number of such patients exceeds 3-4 million people. Since the number of patients with diabetes doubles every 10-15 years, we can talk about the global epidemic of this disease [1]. With the increase in the life expectancy of patients with DM, the new, extremely important issue comes to the forefront and is connected with the growing severity of late complications of the disease that involve practically all organs and systems. Not exception is the damage to the osteoarticular system in patients with DM, the prevalence of which, according to various authors, is from 10 to 77.8% [2,3,4], with the involvement of joints in 58% of patients with type 1 DM and 24% of patients with type 2 DM. However, development, clinical picture and diagnosis of diabetic arthropathies require a comprehensive investigation since it has turned out that only fragmentary studies dedicated to these issues are available today.

Damage to the joints in patients with DM is associated with the occurrence of degenerative-dystrophic changes in the juxta-articular structures, vessels and bone tissues [5]. DM contributes to the development of the biochemical preconditions for the formation of a clinical picture of joint damage [6].

The only energy substrate for chondrocytes with an anaerobic metabolism is glucose, the content of which in chondrocytes is much lower than in synovial fluid and plasma. Delivery of glucose to chondrocytes is carried out by transport proteins GLUT1 and GLUT3 without participation of insulin [7].

Insufficient supply of glucose to chondrocytes, including those with diabetes, leads to a decrease in the intensity of synthetic processes and primary degeneration of cartilage tissue. At the same time, chronic hyperglycemia with type 2 DM inevitably leads to an increase in glucose concentration in synovial fluid, ligaments and joint capsules, which causes osteoarthrosis in type 2 DM through activation of the polyol pathway of glucose metabolism and non-enzymatic glycation of proteins. [8.9]. The formation of end-products of glycation (AGEs), in turn, stimulates chondrocytes and synoviocytes to produce prodegenerative (destructive) and pro-inflammatory mediators and alter the quality of subchondral bone tissue [10]. Neurotoxicity of hyperglycemia leads to neuromuscular insufficiency, which also aggravates joint damage, resulting in destabilization of the joint and worsening of degenerative-dystrophic changes in it [11].

Diabetic arthropathy is characterized by synovial

inflammation that occurs with increased expression of proinflammatory mediators and accelerated catabolism of the matrix of articular cartilage. Synovitis activates sensory nerve fibers as well as causes pain and neurogenic inflammation [12,13]. That is, the imbalance of cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-1, 6, 17) plays a crucial role in the development of a chronic inflammatory disease. Simultaneously, chondrocytes express receptors for IL-1, which increase their sensitivity to this cytokine [14]. The function of IL-1 is its effects on plasminogen, which promote the transformation of the latter into an active plasmin, which, in turn, translates inactive pro-metal proteases into an active form, enhancing the degradation of the extracellular matrix. The catabolic effect of IL-1 manifests itself in its ability to stimulate chondrocytes and synoviocytes to release nitrogen oxide (NO) that damages the extracellular matrix. In addition, NO activates IL-1 and affects apoptosis of chondrocytes by reducing the concentration of an IL-1 receptor antagonist. IL-1β enhances calcium excretion and activates osteoblasts, which leads to a decrease in the intensity of bone formation. IL-1β causes reduced concentrations of osteocalcin, thus promoting the destruction of the subchondral bone [15,16]. Moreover, hyperexpression of the enzyme cyclooxygenase-2, which induces the synthesis of prostaglandins involved in the development of inflammation, is observed. There is good reason to believe that proinflammatory cytokines can be produced by chondrocytes or cells of surrounding tissues, even in the absence of explicit inflammation, while the DM itself initiates increased activity of these cytokines [17]. Thus, expression of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) was observed in the subchondral bone, cartilage and synovial membrane of joints without clinical signs of inflammation. It is known that TNF-α has receptors on chondrocytes, it activates inflammation and tissue damage in joint diseases, stimulates the synthesis of prostaglandins, platelet activation factor, superoxide radicals, metalloproteinases, induces the synthesis of other proinflammatory cytokines (IL-1, -6, -8 and etc.). TNF-α stimulates proliferation of fibroblasts and inhibits the synthesis of collagen and proteoglycans. The described mechanisms of the effect of TNF-α on the components of homeostasis and inflammation of the musculoskeletal system may support degenerative and inflammatory processes in the joints [15,17]. The elderly with obesity and pain in the knee joints had decreased soluble TNF-α receptor while trying to reduce their body weight [18].

IL-6 was high in patients with joint pathology associated with diabetes mellitus, especially in those with obesity. It was found that IL-6 affects resorption of bone tissue, release of matrix metalloproteases, inhibits the synthesis of proteoglycans and collagen by chondrocytes [18].

Thus, proinflammatory cytokines impair the homeostasis of the extracellular matrix of the articular cartilage as well as contribute to the increased levels of superoxide radicals, the synthesis of metalloproteinases and the decreased synthesis of their inhibitors, which ultimately leads to the degeneration of cartilage and the development of inflammatory and degenerative processes of joints in association with diabetes mellitus. In the pathogenesis of joint damage, a wide range of inflammatory mediators is involved, the effect of which extends not only to cartilage, but also to the synovial membrane, subchondral bone, ligament apparatus and results in the development of synovitis, periostitis, tendinitis.

## THE AIM

The aim of our study was to investigate the level of TNF-alpha, IL-1 alpha, IL-6 and receptors for S IL-6-R in patients with diabetic arthropathy and their possible effects on the development of this complication.

## MATERIALS AND METHODS

The study was attended by 118 patients (39 men and 79 women) who were undergoing medical treatment at the State Institution "V. P. Komisarenko Institute of Endocrinology and Metabolism" of NAMSU. Of these, 61 patients were diagnosed with type 1 DM (23 men and 38 women), and 57 patients had type 2 DM (16 men and 41 women). Women were predominant in both groups ($p<0.05$), but gender differences between groups with diabetes mellitus were insignificant ($\chi 2 = 1.2$; $p = 0.26$). The mean age of patients and their body mass index (BMI) are significantly lower than in the group of patients with type 2 DM ($p <0.001$). In this sample, the mean BMI in the group of patients with type 2 DM is 23.0% higher ($t = 13.2$; $p = 0.001$) than in the group of patients with type 1 DM. DM duration is 19.0% higher ($t = 2.3$; $p = 0.023$) in the group of patients with type 1 DM. Gender differences in the observed values were not detected ($p> 0.3$).

Arthropathy was noted in 90 (77.2%) patients, and in 28 (22.8%) patients with DM, this complication was absent. The presence and degree of severity of diabetic arthropathy was evaluated using the Rosenbloom method [19]. Nephropathy was graded according to C. F. Mogenson classification (1992) and retinopathy – according to E. Kohner, M. Porta classification. The content of glucose in the blood was determined by glucose oxidase method. Normal values were considered from 3.3 to 5.5 mmol/l. The degree of compensation of carbohydrate metabolism of the examined patients was assessed by the level of glycosylated hemoglobin (HbA1c), which was determined by calorimetric method with thiobarbituric acid. Compensation for DM was recorded at HbA1c level up to 7%. The research also included traditional clinical tests (biochemical blood test, complete blood count, urinalysis, determination of daily proteinuria, K, Ca total and ionized glycemic profile). The function of the kidneys was evaluated by GFR using the CKD-EPI formula. The content of IL-6, TNF-α, IL-1 and S IL-6-R receptors in serum was determined by the enzyme-linked immunosorbent assay (ELISA) using a set of reagents from Diaclone company (France) and Stat fax 3200 (USA) – ELISA microwell plate reader.

The statistical processing of the findings was carried out

**Table I.** Comparison of mean values of laboratory parameters in patients with type 1 DM and type 2 DM (t-test)

| Parameters | Type 1 DM | | | Type 2 DM | | | t | p |
|---|---|---|---|---|---|---|---|---|
| | X | m | σ | X | m | σ | | |
| HbA1c,% | 8,40 | 0,20 | 1,59 | 8,24 | 0,15 | 1,15 | 0,6 | 0,531 |
| Fasting glucose, mmol/l | 9,89 | 0,51 | 3,98 | 8,03 | 0,28 | 2,11 | 3,2 | 0,002 |
| Blood glucose after a meal mmol/l | 11,44 | 0,59 | 4,62 | 10,39 | 0,41 | 3,06 | 1,5 | 0,149 |
| Cholesterol, mmol/l | 5,46 | 0,16 | 1,24 | 5,85 | 0,23 | 1,72 | 1,4 | 0,169 |
| Creatinine, mkmol/l | 93,56 | 3,69 | 28,85 | 97,56 | 3,81 | 28,75 | 0,8 | 0,452 |
| GFR, ml/min | 107,57 | 6,57 | 51,31 | 90,62 | 5,43 | 40,98 | 2,0 | 0,049 |
| Proteinuria, g/l | 0,08 | 0,02 | 0,13 | 0,15 | 0,03 | 0,25 | 2,0 | 0,047 |
| Calcium, mmol/l | 2,29 | 0,07 | 0,56 | 2,16 | 0,03 | 0,25 | 1,6 | 0,115 |
| Kalium, mmol/l | 4,45 | 0,06 | 0,37 | 4,41 | 0,05 | 0,31 | 0,5 | 0,644 |
| Total protein,g/l | 69,25 | 0,82 | 6,42 | 69,58 | 0,60 | 4,54 | 0,3 | 0,749 |

**Table II.** Mean values of cytokines at the distribution of the patients according to the type of DM and gender (two-factor dispersion analysis)

| Groups | TNF, pg/ml | | | | IL-6 ,pg/ml | | | | IL – alpha, pg/ml | | | | S IL - 6-R, pg/ml | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | X | σ | m | N | X | σ | m | N | X | σ | m | N | X | σ | m |
| Type 1 DM | 60 | 20,2 | 12,4 | 1,6 | 60 | 23,8 | 19,7 | 2,5 | 20 | 12,0 | 3,3 | 0,7 | 20 | 323,7 | 68,9 | 15,4 |
| Men | 23 | 19,7 | 10,2 | 2,1 | 23 | 26,0 | 18,4 | 3,8 | 6 | 11,6 | 2,8 | 1,1 | 6 | 344,5 | 89,4 | 36,5 |
| Women | 37 | 20,5 | 13,7 | 2,3 | 37 | 22,5 | 20,5 | 3,4 | 14 | 12,2 | 3,6 | 1,0 | 14 | 314,8 | 59,8 | 16,0 |
| Type 2 DM | 55 | 24,5 | 17,4 | 2,3 | 55 | 28,6 | 25,2 | 3,4 | 18 | 11,3 | 3,0 | 0,7 | 18 | 358,7 | 64,4 | 15,2 |
| Men | 16 | 23,9 | 23,0 | 5,8 | 16 | 27,3 | 30,2 | 7,5 | 5 | 10,3 | 1,8 | 0,8 | 5 | 373,6 | 79,4 | 35,5 |
| Women | 39 | 24,7 | 14,9 | 2,4 | 39 | 29,2 | 23,2 | 3,7 | 13 | 11,7 | 3,3 | 0,9 | 13 | 353,0 | 60,4 | 16,8 |
| F | 0,00 | | | | 0,36 | | | | 0,11 | | | | 0,04 | | | |
| P | 1,00 | | | | 0,549 | | | | 0,747 | | | | 0,852 | | | |

using the methods of variation statistics of the standard package for statistical calculations Statistica 5.0 Microsoft OfficeExel 2003. The study presents statistical variables of the mean values (denoted as M) and the mean square deviation (SD), the standard error of the average value (m). To compare mean absolute values, the t-criterion of Student was used in the various study groups. The difference in the findings was considered statistically significant with the value of the index $p \leq 0.05$. Correlation, dispersion, single-factor and multiple regression analysis, as well as discriminant statistics were used for data analysis.

## RESULTS

In the group of patients with type 1 DM, arthropathy was detected in 75.4 ± 5.5% of patients, in the group of patients with type 2 DM – in 77.2 ± 5.6% (t = 0.23; p> 0.1). This sample did not confirm the assumption that women with type 2DM have higher chances of developing arthropathy than men (OR = 2.91; CI = 0.80-10.68; p = 0.106). In type 1 DM, the chances of developing arthropathy in men and women are also equal (OR = 1.14; CI = 0.34-3.75; p = 0.833). In the group of patients with type 1 DM, kidney damage was detected in 72.9% (n = 35) of patients and with type 2 DM – in 74.5% (n = 38) of patients. Arthropathy was diagnosed in 66,7% (n = 22) of patients with type 1 DM and kidney damage and in 81,6% (n = 31) of patients with type 2 DM and kidney damage. The relationship between the factors of kidney damage and the development of arthropathy has been confirmed only for patients with type 2 DM. The chances for the development of arthropathy in patients with type 2 DM and renal impairment are 5.2 times higher than in patients without this complication (OR = 5.17; CI = 1.32-20.22; p = 0.018). For patients with type 1 DM, the chances are equal (OR = 0.31; CI = 0.06-1.61; p = 0.163).

In the group of patients with type 2 DM, fasting glucose levels and GFR in the blood are significantly lower than in the group of patients with type 1 DM (Table I). The level of protein in urine is higher in the group of patients with type 2 DM. According to other laboratory parameters, no statistical differences were detected.

Comparison of mean values of laboratory parameters in patients with and without arthropathy and in patients with different types of DM showed an increase in the level of GFR in patients with type 1 DM and arthropathy (t = 2.2; p = 0.034). In patients with type 1 DM without arthropathy, the GFR rate is 88.73 ± 7.96 ml / min, with arthropathy – 113.72 ± 8.15 ml / min. The corresponding rates in the group of patients with type 2 DM are 95.63 ± 9.11 and 89.15 ± 6.53 ml / min. Other indicators of statistical difference are

not defined. An increase in the level of GFR may indicate a compensatory reaction of the kidneys in the initial stages of nephropathy, and as it is known, arthropathy is an advanced complication of DM, and more often develops in patients with DM complicated by other conditions.

A two-factor dispersion analysis with the factors "DM" and "gender" did not reveal any differences in the average levels of cytokines (Table II). Logistic regression analysis found out that in diabetes mellitus and the presence of arthropathy, the levels of tumor necrosis and interleukin-6 are significantly higher than in the absence of arthropathy. Logistic regression models with a dependent variable of TNF and the presence / absence of arthropathy as an independent variable are statistically significant both for the group of patients with type 1 DM ($\chi2 = 27.2$; $p < 0.001$) and for the group of patients with type 2 DM ($\chi2 = 26.8$; $p < 0.001$). The sensitivity of the model for a group of patients with type 1 DM was 53.3%, specificity – 93.0%. The corresponding rates for the group of patients with type 2 DM were 76.9% and 90.5%. With an increase in the level of TNF in type 1 DM, the chances for the detection of arthropathy rise 1.7 times (OR = 1.70; CI 1.19-2.44), in type 2 DM – 1.8 times (OR = 1.78; CI 1.21-1.2.61).

Logistic regression models with dependent variable IL-6 and the presence / absence of arthropathy as an independent variable are also statistically reliable for the group of patients with type 1 DM ($\chi2 = 23.2$; $p < 0.001$) and for the group of patients with type 2 DM ($\chi2 = 29.6$; $p < 0.001$). The sensitivity of the model for the group of patients with type 1 DM was 70.0%, specificity – 90.6%. The corresponding rates for the group of patients with type 2 DM were 92.3% and 86.2%. With an increase in the level of IL-6 in type 1 DM, the chances for the detection of arthropathy rise 1.5 times (OR = 1.47; CI 1.08-1.98), in type 2 DM – 1.3 times (OR = 1.34; CI 1.03-1.74).

Reliable logistic regression models with IL-1 IL-6R as independent variables and the presence / absence of arthropathy as an independent variable could not be obtained ($p > 0.2$).

Differences in the average level of TNF were determined depending on the stage of arthropathy ($F = 37.3$; $p < 0.001$). In patients with type 1 DM, the average level of TNF in the presence of arthropathy is 44.5% higher than in its absence ($t = 5.2$; $p < 0.001$). For type 2 DM, this value is 42.9% ($t = 7.2$; $p < 0.001$). In the case of type 1 DM, there were identified some differences in the mean values of TNF in patients without arthropathy and with the 1st stage ($t = 3.1$; $p < 0.01$), and with the 2nd and 3rd stages ($t = 2.4$; $p < 0.05$). No significant differences were found between the levels of TNF at the 1st and 2nd stages ($t = 1.7$; $p = 0.10$). In the case of type 2 DM, there were established some differences in the mean values of TNF in patients with the 1st and 2nd stages ($t = 3.6$; $p < 0.01$) and with the 2nd and 3rd stages ($t = 4.4$; $p < 0.001$). No significant differences were found between the levels of TNF in the absence of arthropathy and the 1st stage ($t = 0.96$; $p = 0.36$).

There were determined some differences in the average level of IL-6 depending on the stage of arthropathy ($F = 28.2$; $p < 0.001$). In patients with type 1 DM, the average level of IL-6 in the presence of arthropathy is 52.1% higher than in its absence ($t = 5.6$; $p < 0.001$). For type 2 DM, this value is 64.4% ($t = 7.3$; $p < 0.001$). In type 1 DM, there were detected some differences in the mean values of IL-6 in patients without arthropathy and with the 1st stage ($t = 3.4$; $p < 0.05$) and with the 1st and 3rd stages ($t = 2.8$; $p < 0.05$). No significant differences were found between the levels of IL-6 at the 1st and 2nd, as well as the 2nd and 3rd stages ($t = 1.8$; $p = 0.07$ and $t = 1.9$; $p = 0.08$). In type 2 DM, there were detected some differences in the mean values of IL-6 in patients without arthropathy and with the 1st stage ($t = 2.6$; $p < 0.05$), with the 1st and 2nd stages ($t = 3.9$; $p < 0.01$) and with the 2nd and 3rd stages ($t = 3.4$; $p < 0.01$). For IL-1 and IL-6 R, differences in mean values at different stages of arthropathy were not detected ($p > 0.4$).

## DISCUSSION

Thus, the development of joint damage in patients with diabetes of both types is accompanied by increased levels of pro-inflammatory cytokines. IL-6, TNF-α were the most sensitive for patients with diabetes with joint lesions. Our results are confirmed in many studies, because these cytokines are most often mentioned as inducers of nonspecific inflammation and stimulators of the synthesis of other interleukins, which promote cartilage catabolism through the synthesis of metalloproteases, induction of oxidative stress and apoptosis of chondrocytes [15, 20]. Although it is known that the main trigger of joint degradation in patients with diabetes is hyperglycemia [12]. And in our study, the vast majority of patients were in a state of decompensation (HbA1c level exceeded 8%). Local increase in glucose concentration leads to changes in cartilage tissue due to increased end products of increased glycosylation (AGEs), which, in turn, stimulates chondrocytes and synoviocytes to produce prodegenerative (destructive) and proinflammatory mediators [21]. The level of pro-inflammatory cytokines is a reflection of nonspecific inflammation. A probable increase in IL – 6, TNF-α was found in patients with type 1 and type 2 diabetes with arthropathies. In patients with type 1 diabetes, the average level of IL-6 in the presence of arthropathy is 52.1% higher than in its absence, in patients with type 2 diabetes – by 64.4%. In the analysis of this indicator, depending on the stage of arthropathy, a probable increase in IL-6 with each stage of the disease in patients with diabetes of both types with arthropathy. In the examined patients with type 1 diabetes, the average level of TNF-α in the presence of arthropathy is higher by 44.5% than in its absence, in type 2 diabetes – by 42.9%. TNF-α has receptors on chondrocytes, is an activator of inflammation and tissue damage in OA, stimulates the synthesis of prostaglandins, platelet activating factor, superoxide radicals, metalloproteinases, induces the synthesis of other proinflammatory cytokines (IL-1, -6, -8 and others). TNF-α stimulates the proliferation of fibroblasts and inhibits the synthesis of collagen and proteoglycans. The biological action of TNF-α as an inflammatory cytokine depends on

its concentration in tissues. At high concentrations, which we obtained in our study, TNF-α acts as a mediator of cartilage and bone damage and the development of systemic inflammatory reactions [16,22].

In our work, reliable logit regression models with IL-1 and IL-6R as independent variables and the presence / absence of arthropathy as an independent variable could not be obtained. Although, it is believed that the increase in IL-1 is the most characteristic of osteoarthritis [15, 20, 21, 22]. But almost all work concerned IL-β. It is known that IL 1 is one of the pathogenetically significant cytokines in OA. IL 1 itself increases the transcription of genes that control destructive processes in cartilage and the production of proinflammatory prostaglandins. As a result – suppression of chondrocyte production by type 2 collagen and aggrecans. In addition, the level of IL-α reflects to a greater extent the lesions of the large supporting joints of the lower extremities (hip, knee) [23]. Because, in our study, the vast majority of affected joints affected the upper extremities, this may be due to the lack of probable IL-α changes in patients with arthropathy. Thus, the most informative markers among cytokines, according to our studies and IL-6, TNF-α.

## CONCLUSIONS

1. It has been established that women and men with type 1 and type 2 DM have similar chances for the development of arthropathy.
2. In patients with type 1 DM and arthropathy, an increase in the level of GFR was noted. It may indicate a compensatory reaction of the kidneys in the initial stages of nephropathy, and as it is known that arthropathy is an advanced complication of DM, and more often develops in patients with DM complicated by other conditions. Although the chances of developing arthropathy for patients with type 1 DM and renal impairment were equal (OR = 0.31; CI = 0.06-1.61; p = 0.163), the chances for the development of arthropathy in patients with type 2 DM and renal impairment were 5.2 times higher than in patients without this complication (OR = 5.17; CI = 1.32-20.22; p = 0.018).
3. In patients with type 1 DM, the average level of TNF in the presence of arthropathy is 44.5% higher than in its absence (t = 5.2; p <0.001). For type 2 DM, this value is 42.9% (t = 7.2; p <0.001). With an increase in the level of TNF in type 1 DM, the chances for the detection of arthropathy rise 1.7 times (OR = 1.70; CI 1.19-2.44), in type 2 DM – 1.8 times (OR = 1.78; CI 1.21-1.2.61).
4. In patients with type 1 DM, the average level of IL-6 in the presence of arthropathy is 52.1% higher than in its absence (t = 5.6; p <0.001). For type 2 DM, this value is 64.4% (t = 7.3; p <0.001). With an increase in the level of IL-6 in type 1 DM, the chances for the detection of arthropathy rise 1.5 times (OR = 1.47; CI 1.08-1.98), in type 2 DM – 1.3 times (OR = 1.34; CI 1.03-1.74).
5. For IL-1 and IL-6 R, differences in mean values at different stages of arthropathy were not detected (p> 0.4).
6. As a result, the main proinflammatory cytokines, namely, TNF-alpha and IL-6, which may serve as markers of the presence and progression of arthropathy in patients with diabetes mellitus, can be identified.

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*The Authors declare no conflict of interest.*

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ORIGINAL ARTICLE

# MARKETING AUDIT OF UKRAINIAN PHARMACEUTICAL MARKET FOR LOCAL TREATMENT ACNE AND DEMODICOSIS

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## ABSTRACT

**The aim**: To conduct a marketing audit of the pharmaceutical market of Ukraine for acne and demodicosis medications.

**Materials and methods:** The study used marketing methods of analysis, survey, observation methods and reference literature – State Register of Medicines of Ukraine of the State Expert Center of the Ministry of Health of Ukraine.

**Results:** As a result of marketing audit of retail audit data of pharmacy sales revealed that the leading sales in packaging is MNL benzylbenzoate, the share of which reached in different years from 45% to 32%, which was 1380902 and 647576 packages per year, respectively, in second place – metronidazole , benzoyl peroxide, zinc ointment and sulfur ointment respectively.

**Conclusions:** According to our analysis, it has been found that the highest sales volume (in packs) are of the low-cost brands, which are the most economically available and have the most effective therapeutic effect. Therefore, it is important to create multicomponent soft drugs in the form of cream and gel based on metronidazole, benzyl benzoate and benzoyl peroxide.

**KEY WORDS:** sales dynamics, local therapy, acne, demodicosis, cream, gel, ointment

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## INTRODUCTION

Acne (*acne*), demodicosis, rosacea, perioral dermatitis, belonging to a special group of skin diseases, psychosomatic dermatoses, is a polymorphic multifactorial disease of the sebaceous glands. The term «acne» emphasizes the chronic, often recurrent course of dermatosis, the complexity of its etiopathogenesis, and the need for a comprehensive approach to the treatment of the disease [1-3] . Currently, the incidence of acne and demodicosis not only has no tendency to decrease, but also significantly increases. According to the scientific organization of the European Dermatological Forum, the disease of demodicosis can go into acne. Given the localization of rashes on the face in almost all patients, there is no doubt that acne and demodicosis have an effect on their psycho-emotional state [4-6]. Thus, the pronounced impact of the disease on the psycho-emotional sphere and social adaptation of patients determine the urgency of this problem and the need to develop new effective treatments. In this regard, the development of drugs for local therapy of this disease is an urgent medical and pharmaceutical task.

## THE AIM

The aim of this study was to conduct a marketing audit of the Ukrainian pharmaceutical market for the treatment of dermatological diseases such as acne and demodicosis and to identify whether there are registered multicomponent drugs and the level of realization of which active pharmaceutical ingredients are most in demand.

## MATERIALS AND METHODS

In order to evaluate the prospect of developing new drugs for local acne therapy and demodicosis, we conducted a study to investigate existing demand and consumer trends in the soft drug segment of the relevant area. The study used marketing methods of analysis and reference literature – State Register of Medicines of Ukraine of the State Expert Center of the Ministry of Health of Ukraine, survey and observation methods, as well as retrospective analysis of the retail audit of the pharmaceutical market of Ukraine for the last 10 years by international non-patented names (INN) drugs and medicines audit for the treatment of dermatological diseases, in particular acne and demodicosis according to ICD 10.

## RESULTS AND DISCUSSION

The analysis was aimed at studying the drugs in the domestic pharmaceutical market by groups P03A, D06BX, D10AD, D10A, D06A, D02A according to the anatomical-therapeutic chemical classification (ATC).

Medicinal products for the treatment of acne and demodicosis according to ATC-classification are shown in Table. I

According to the table. 1 for the treatment of acne and demodicosis apply 6 groups of drugs (on ATC), of which 2 – (P03A, D06BX), acting on ectoparasites, including scabies, antimicrobials and 4 groups (D10AD, D10A, D06A , D02A) medicines – retinoids for topical treatment of acne, antibiotics for topical use, drugs with softening and protective effects.

**Table 1.** ATC classification of drugs for the treatment of acne and demodicosis

| ATC code 3-4 level | ATC code 5 level | Commercial name | API |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| P03A – Means acting on ectoparasites, including scabies | P03AX01 - Benzyl benzoate<br>P03AC04 - Permethrin | Benzyl benzoate<br>Permethrin<br>Permethrin ointment<br>Permin | Benzyl benzoate<br>Permethrin |
| D06BX - Antimicrobials other | D06BX01 -Metronidazole | Metrogyl<br>Rosamet | Metronidazole |
| D10AD - Retinoids for local acne treatment | D10AD04 - Isotretinoin | Derivia water<br>Deriv C MS | Adapalene<br>Micronized clindamycin |
| D10A – Local remedies for acne treatment | D10AX - Other medicines for local acne treatment | Acne stope<br>Curiozin<br>Skinoren | Azelaic acid<br>Zinc is hyaluronate<br>Azelaic acid |
| D10A – Local remedies for acne treatment | D10AE01 - Benzoyl peroxide | Ugresol | Benzoyl peroxide |
| D10A – Local remedies for acne treatment | D10AB02 - Sulfur | Simple Sulfur Ointment | Sulfur |
| D10A – Local remedies for acne treatment | D10AF - Antimicrobial medicines for the treatment of acne | Duak<br>Zerkalin<br>Zinerit | Benzoyl peroxide + clindamycin<br>Clindamycin<br>erythromycin, zinc acetate |
| D06A - Antibiotics for local use | D06AX01 - Fusidium acid | Fuziderms<br>Fuzykutan | Fusidium acid |
| D02A-Preparations with softening and protective action | D02AB - Zinc preparations | Zinc ointment<br>Sudokrem | Zinc is oxide<br>benzyl benzoate, zinc oxide, benzyl cinnamate, benzyl alcohol |

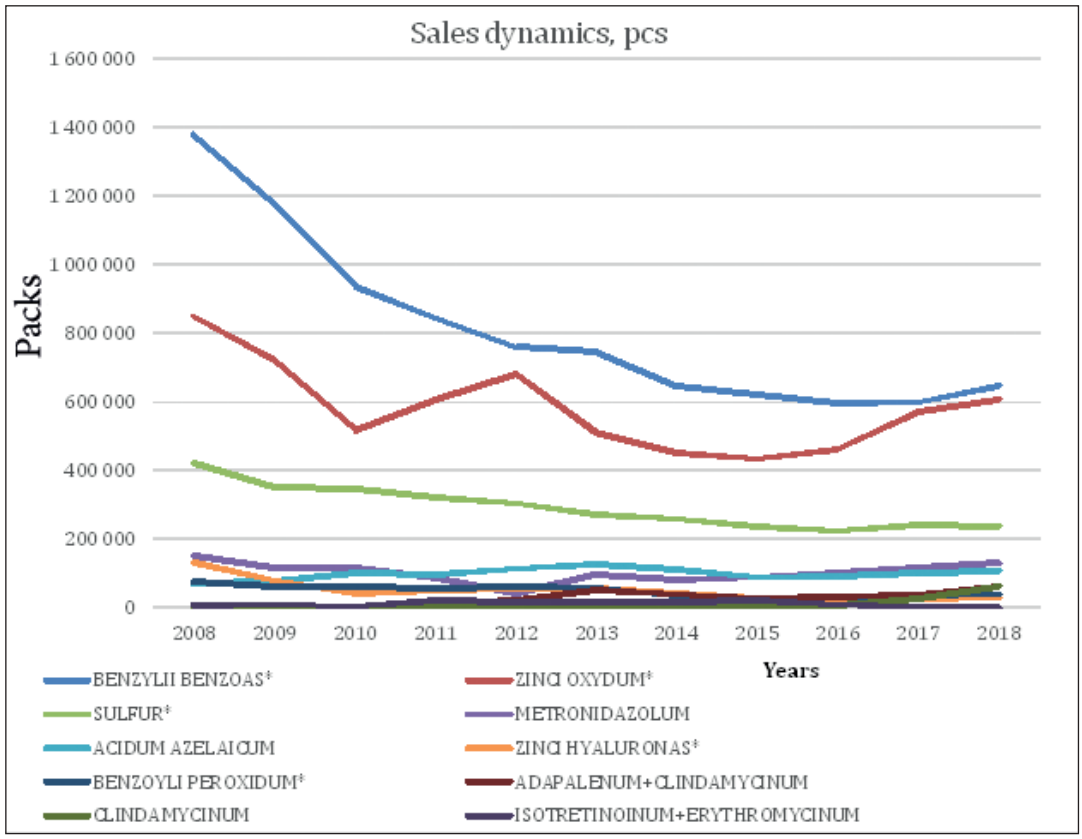


**Fig. 1.** Dynamics of pharmacy sales of local medicines for the treatment of acne and demodicosis of the medicines groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A)

Data analysis Fig. 1 showed that the market share of the relevant INNs can be observed, with regard to consumer preferences remain quite stable for a long time, we see a small increase in the market share of zinc ointment (D02AB), metronidazole (D06BX01) and clindamycin (D10A), which however does not change the list and

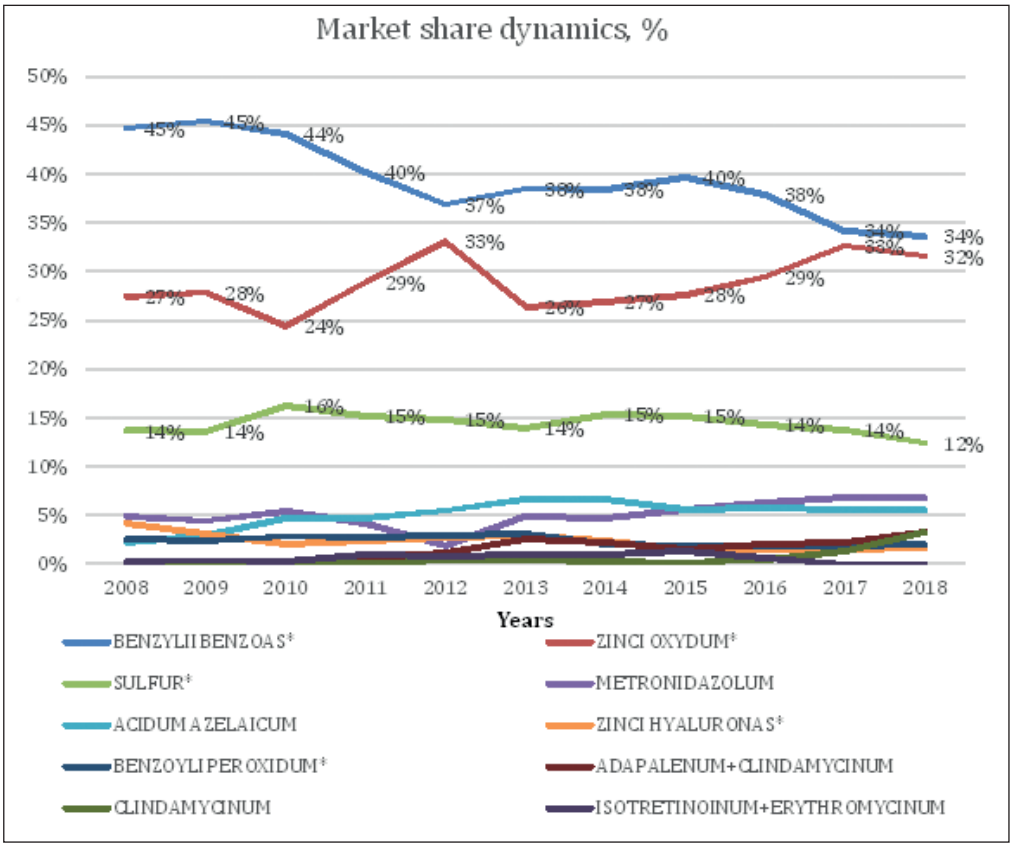


**Fig. 2.** Dynamics of Local Pharmaceutical Sales (LZ) Market Share for Acne and Demodicosis treatment of medicines groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A)

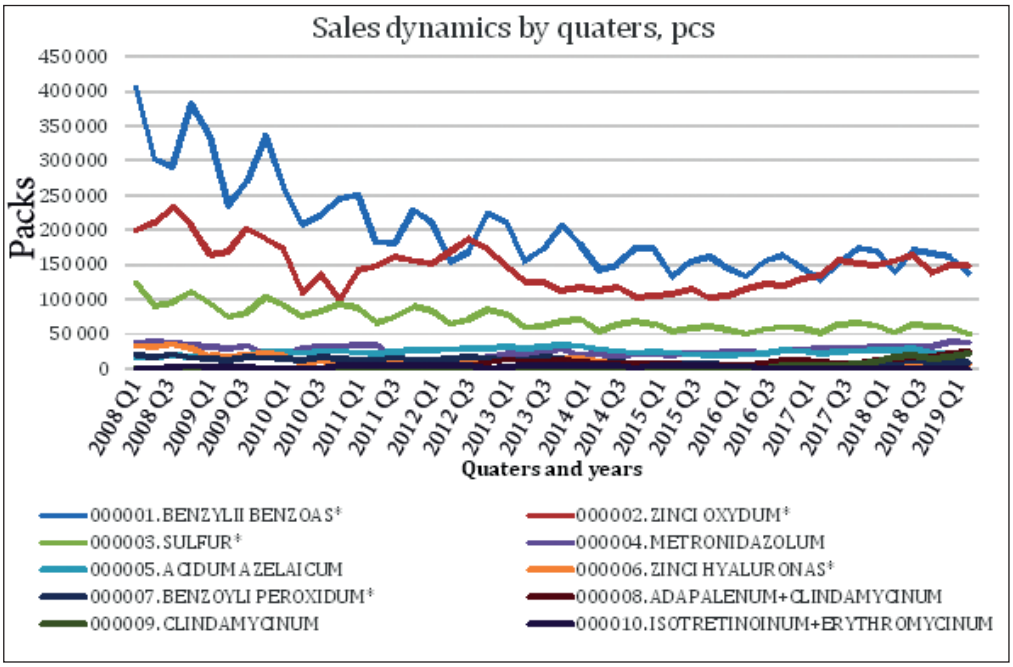


**Fig. 3.** Dynamics of pharmacy sales quarterly, local medicines for the treatment of acne and demodicosis of the medicines groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A)

popularity segment leaders. In order to develop hypotheses about the reasons for such distribution and the sustainability of consumer preferences and the influence of various factors on this, we conducted a thorough analysis of sales (Fig. 2).

Data analysis Fig. 2 showed that quarterly sales dynamics were evaluated to test the market seasonality hypothesis. Benzylbenzoate-based preparations (P03AX01) and sulfur (D10AB02) have some third-quarter sales growth and a decline in the second. Zinc formulations (D02AB) show a multidirectional trend in different years, but preferably also a slight increase in Q3. Other international non-branded name do not show pronounced seasonal dynamics. The reasons for such differences for different international non-branded name may be the following: on small sales, seasonal differences are less noticeable; additional indications that these international non-branded name are used – for example, zinc ointment

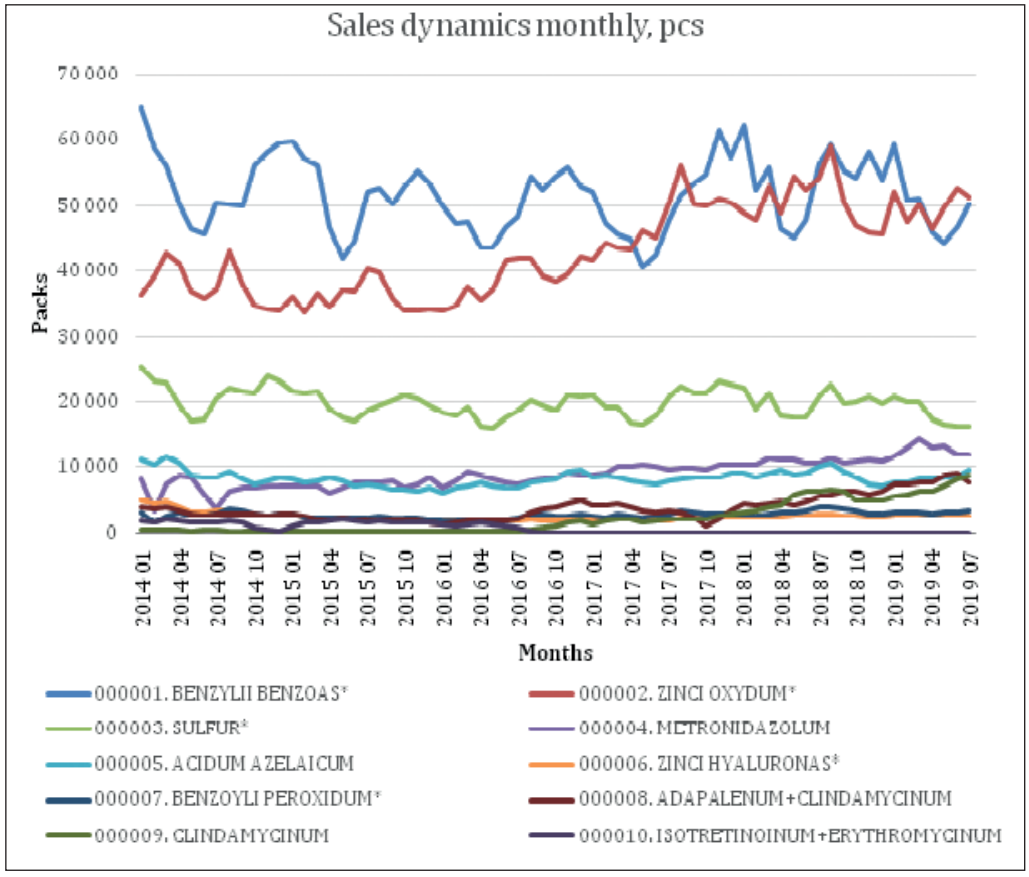


**Fig. 4.** Dynamics of pharmacy monthly sales of local medicines for the treatment of acne and demodicosis of drug groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A)

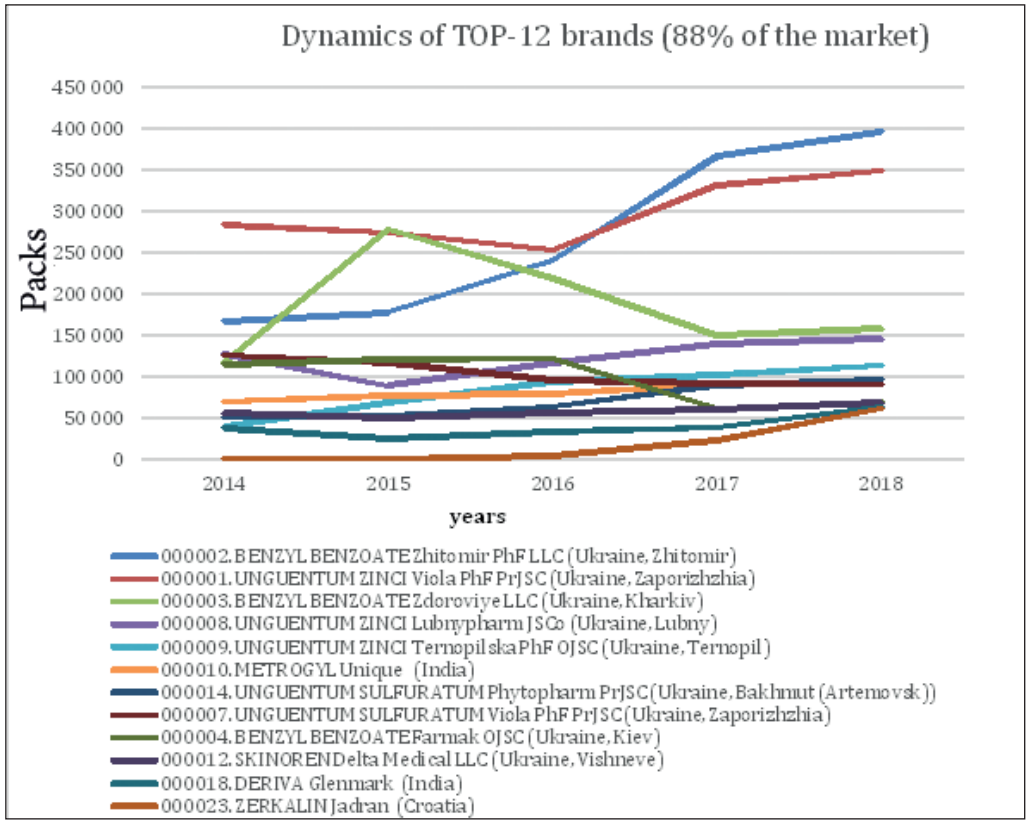


**Fig. 5.** Dynamics of the Top 12 Local Medicinal Products (LZ) Brands for Acne and Demodicosis of medicines groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A)

(D02AB), in addition to acne and demodicosis, are also used to treat diaper dermatitis and rashes in infants where seasonality is not expressed and sulfur (D10AB02) and benzyl benzoate (P03AX01) have in scabies, which is more common in autumn and winter. But if you look at the seasonality of acne (mainly autumn and winter)

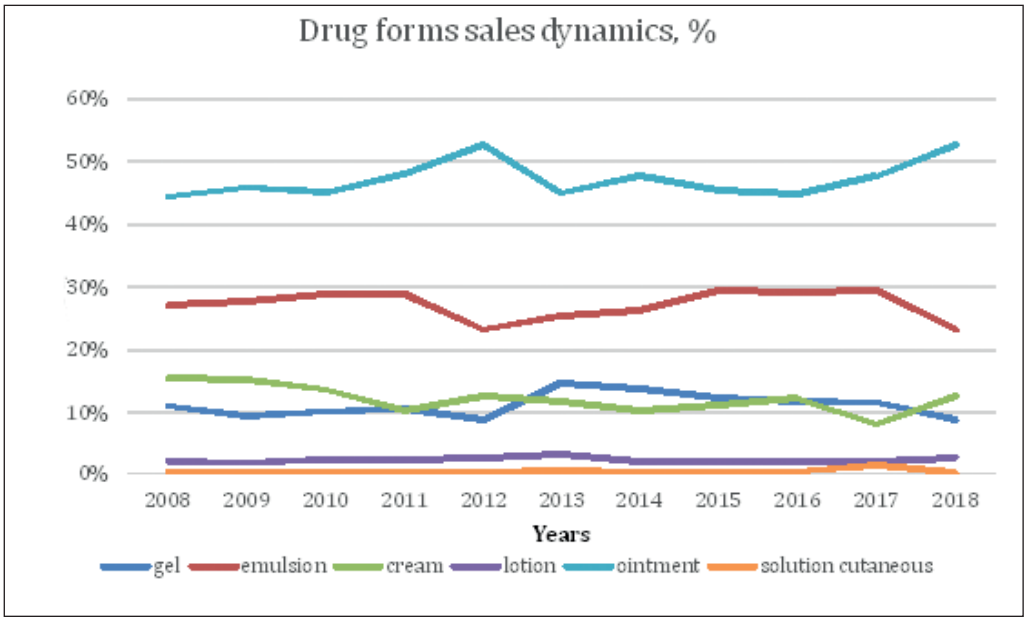


**Fig. 6.** Proportion of various dosage forms in the topical segment for the treatment of acne and demodicosis of the medicines groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A)

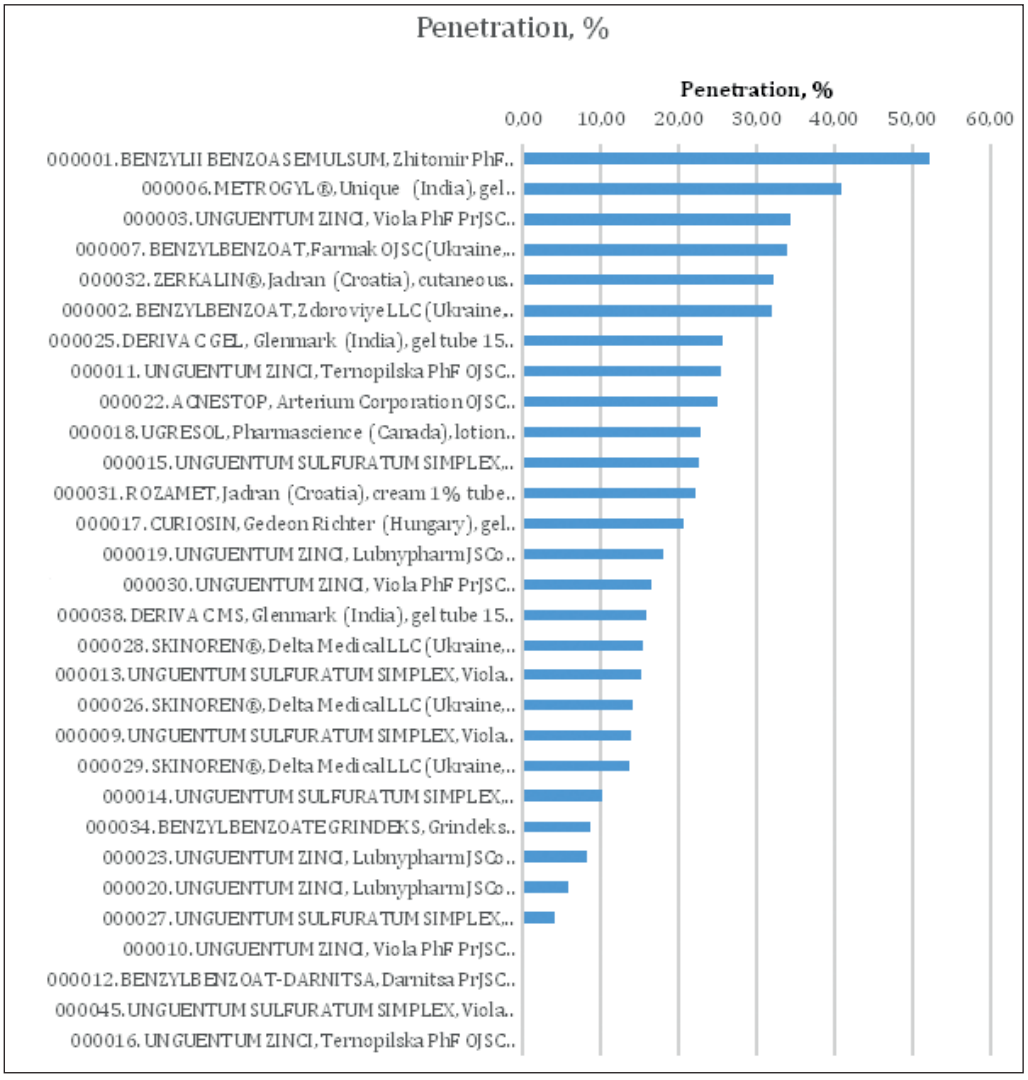


**Fig. 7.** Penetration of sales

and demodicosis (bursts from May to September), we will notice that the seasonal fluctuations in sales of the 3 most popular international non-branded name just cover these periods.

According to Fig. 3, the monthly breakdown of sales showed an upward trend in the groups (P03A, D06BX) and (D10AD, D10A, D06A, D02A), starting in May and the marked growth of the groups (P03AX01) and (D10AB02) in the autumn.

Figure 4 presents the monthly dynamics of sales in packages.

An assessment of the dynamics of individual brands (Fig. 4) found that 88% of the market (in packaging) is occupied by sales of 12 brands. There have been some changes among benzyl benzoate (P03AX01), sulfur (D10AB02) and zinc (D02AB) based products from different manufacturers – some manufacturers have ceded to other positions. Thus, among benzylbenzoate (P03AX01) preparations, the benzylbenzoate emulsion produced by ZPP Pharmaceutical Factory LLC, Zhytomyr, which is today the leader in the segment as a whole and the best-selling brand among benzylbenzoates, has also grown. production of JSC Pharmaceutical factory «Viola» and JSC Lubnifarm, sulfur ointment (D10AB02) of production of JSC «Phytopharm» and others[6, 7].

The next stage of our research was (Fig. 5) conducting an analysis of the popularity of various dosage forms in the segment of external agents used to treat acne and demodicosis. The dynamics of sales in packaging and the proportion of each dosage form in the segment were evaluated. It is proved that the most common forms are ointment and emulsion – this correlates with the leading drugs in packaging sales. Thus, in the form of ointments are primarily zinc (D02AB) and sulfur ointment (D10AB02), and in the form of emulsions – benzyl benzoate (P03AX01), which in some manufacturers is also in the form of cream. Ointments occupy about half of the segment in packages (45-50%), emulsions – 23-29% in different years[6, 7, 8].

Forms of cream and gel occupy about 10-13% each and are represented by preparations based on metronidazole (D06BX01), benzoyl peroxide (D10AE01) and the like. Liquid dosage forms (lotions, solutions) occupy the smallest proportion of the segment (in packs).

In the studied market segment (data for July 2019), benzylbenzoate (P03AX01), Zhytomyr CFC – 52.35%, Metrogil (Unic Pharmaceutical Laboratories) – 40.99%, Zinc Ointment (PJSC Pharmaceutical Factory) have the highest prevalence »- 34,5%, Benzylbenzoate (Pharmak JSC) – 34,09%, Zerkalin (JSC« Jadran »Galenskaya laboratory etc.») – 32,22%, Benzylbenzoate («Pharmaceutical company« Health » ») – 32.18%. These drugs the patient can find in every 2-3 pharmacies. Other drugs are presented only in every fourth pharmacy and less frequently.

It is proved that a high penetration rate does not always correlate with a higher rate of sales in packages (Fig. 7). It can be influenced by various factors – price, age and brand awareness, the drug is prescribed mainly by a doctor or the patient chooses it independently or on the recommendation of a pharmacist directly in the pharmacy, the presence of active promotion of manufacturers, the availability of their own brands of pharmacies and other features of distribution. and more[8].

The next indicator is the number of retail outlets (out of the total number of pharmacies in Ukraine) that sold the drug at least once a month (data for July 2019). The highest percentages are found to be in high demand – benzylbenzoate and zinc ointment from various manufacturers. Almost every fourth pharmacy sells metrogill monthly (almost the only form of metronidazole for external use on the market) and Zerkalin. Compared to the sales of TOP-12 brands in the segment, it is proved that these funds either have consistently high sales figures or their sales have been increasing in recent years. The drugs at the bottom of the list are predominantly negative sales dynamics or belong to a higher-end segment or are predominantly prescribed by specialist dermatological venereologists, and thus are sold predominantly at pharmacies near their respective health care facilities.

## CONCLUSIONS

1. As a result of marketing audit of retail audit data of pharmacy sales revealed that the leading sales in packaging is MNL benzylbenzoate, the share of which reached in different years from 45% to 32%, which was 1380902 and 647576 packages per year, respectively, in second place – metronidazole , benzoyl peroxide, zinc ointment and sulfur ointment respectively.
2. As a result of our marketing audit of the domestic pharmaceutical market for Group D drugs, we have found that the range of medicines for topical acne and demodicosis therapy is mainly mono-component drugs.
3. According to our analysis, it has been found that the highest sales volume (in packs) are of the low-cost brands, which are the most economically available and have the most effective therapeutic effect. Therefore, it is important to create multicomponent soft drugs in the form of cream and gel based on metronidazole, benzyl benzoate and benzoyl peroxide.

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ORIGINAL ARTICLE

# THE ROLE OF LIPOPROTEIN (A) AND PREGNANCY ASSOCIATED PLASMA PROTEIN A IN DIAGNOSTICS CORONARY HEART DISEASE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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## ABSTRACT

**The aim:** To identify the significance of biomarkers characterizing the role of lipid disorders and the processes of destruction atherosclerotic plaque for the early diagnosis of CHD in patients with COPD.

**Materials and methods**: There were examined 153 patients, men aged 40-70 years, including 53 patients with COPD, 56 with a combination of COPD and CHD and 44 patients with stable CHD. The level of LP (a) and PAPP-A in the serum was determined by ELISA.

**Results**: There was increased level of LP (a) and PAPP-A in patients with CHD and with a combination of COPD and CHD. This increased level of LP (a) and PAPP-A was associated with the level of C-reactive protein. The mid level of LP (a) and PAPP-A in patients with COPD did not significantly differ from the reference values.

**Conclusions**: The increase level of lipoprotein (a) more than 18 mg/dl in patients with COPD may be regarded as a predictor of the development of CHD. The level PAPP-A more than 5 mIU/L in plasma of patients with COPD makes it possible to isolate the groups for CHD risk. The definition of LP (a) and PAPP-A in patients with COPD may contribute to the early diagnostics of coronary heart disease in the absence of its pronounced clinical manifestations.

**KEY WORDS:** comorbidity of COPD and CHD, lipoprotein (a) (LP (a)), pregnancy-associated plasma protein A (PAPP-A), C-reactive protein

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## INTRODUCTION

Coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD) are among the leading diseases in the world that contributing to their frequent combination. According to J.A. Falk et al. [1] comorbidity of CHD and COPD is from 18.7% to 88.3% in morbidity structure of patients over 40 years old. The combination of COPD and CHD increases the chances of a fatal outcome [2,3,4,5] and leads to the changes of the clinical picture and difficulty in diagnosing CHD [6,7]. Stress tests, ECG, daily monitoring of ECG, echocardiography, myocardial scintigraphy have not a sufficient level of specificity and sensitivity in the diagnosis of myocardial ischemia in patients with COPD, which determines the need further improvement of diagnostic methods taking into account the pathogenetic mechanisms of the disease.

Numerous studies have revealed essential significance in the development of CHD such indicators of blood lipid spectrum as cholesterol low-density lipoproteins (LDL cholesterol), high-density lipoprotein (LDL cholesterol), apolipoprotein Al [8,9]. It has been shown that the risk of developing CHD continuously increases over the whole range values of serum cholesterol [10]. However, at the same time in clinical practice, there are quite often patients with severe forms of CHD, which have a significant part of coronary events occur at normal levels of LDL- cholesterol and HDL- cholesterol in the blood [11]. The results of epidemiological studies indicate that even after reaching the target level of LDL cholesterol, there remains a significant residual risk for which can answer other, less studied, factors, for example, lipoprotein (a) (LP (a)) [8,12,13,14]. LP (a) is actively involved in the formation and growth of atherosclerotic plaque, stimulating the movement of monocytes, the oxidation of LDL and the capture of oxidized LDL by macrophages and promotes the growth of smooth muscle cells. However, in some studies, the relationship of LP (a) with the occurrence of CHD and its complications has not been established [15,16]. A sufficient amount of works has been devoted to the study of the lipid profile in patients with CHD and COPD. But, researches of the lipid profile in patients with a combination of COPD and CHD are rare and ambiguous. And we did not find researches of LP (a) and PAPP-A in patients with combination of CHD and COPD.

It is known that vascular inflammation is the main factor contributing to the progression of the atherosclerosis from the stage of atheroma's formation to the development of destructive changes and formation of a thrombus in the lumen of the coronary artery [17,18,19]. Analysis of inflammatory reactions in the vascular wall showed that the elements of

the vascular immune response could not only trigger atherogenesis, but also modulate and control it [20]. In this regard, in recent years, great interest is caused by the detection in the blood of biochemical substances, the concentration of which reflects the activity of vascular inflammation and endogenous destruction in atherosclerotic plaques. It has been established that the activation of inflammation increased the proteolytic activity of macrophages with the participation of metalloproteinases, that leads to the decrease in the thickness and strength of the fibrous plaque cap that separates blood from the high-thrombogenic substances of the lipid core [21]. There are separate studies in which it was shown that the most promising marker of endogenous destruction of an atheroma can be zinc-containing matrix metalloproteinase, pregnancy associated plasma protein A (PAPP-A) [22,23]. PAPP-A is secreted by activated macrophages that are involved in the local vascular inflammatory process and thus contribute to the development of atherosclerotic lesions and damage of atheroma [24].

Researches of changes the diagnostic and practical significance level of PAPP-A in patients with CHD are not numerous. Most studies have found an increase PAPP-A in patients with unstable atherosclerotic plaques and/or with recent heart attack [25,26]. At the same time, patients with stable CHD more often have normal or slightly elevated PAPP-A level. In this regard, it has been suggested that PAPP-A can be a perspective marker of atherosclerotic plaque damage, it's instability and serve as a predictor of an unfavorable prognosis of patients with CHD [23,27]. Nonetheless, some studies noted that PAPP-A levels can be increased in patients with stable CHD, and elevated concentrations of PAPP-A are associated with adverse heart events [28]. It was noted that PAPP-A levels in the blood with stable CHD were higher in patients with multi-vascular lesion and with complicated stenosis in the coronary arteries, according to coronary angiography, as well as with significant depression of the ST segment, according to the ECG. That is why we may assume that PAPP-A can play the role of an indicator of the presence of hemodynamically significant stenoses in the coronary arteries in patients with stable CHD [29].

A. Elesber et al. [30] found that in patients with stable CHD, the prognostic value of PAPP-A did not depend on the presence of traditional risk factors for coronary atherosclerosis or the magnitude of the ejection fraction, while the relationship between PAPP-A and the development of death or repeated acute coronary syndrome was significant even after correction existing atherosclerosis risk factors. This provided grounds for considering PAPP-A as an independent and informative marker of the risk of an atherosclerotic plaque damage [31]. Data about the study of the PAPP-A level in patients with a combination of CHD and COPD are absent.

Thus, the literature suggests that the combination of COPD and CHD has a high prevalence, but the main causes, mechanisms of occurrence and relationship of these pathologies, diagnostic and prognostic approaches require further study.

## THE AIM

To identify the significance of biomarkers characterizing the role of lipid disorders and the processes of atherosclerotic plaque destruction for the early diagnosis of CHD in patients with COPD.

## MATERIALS AND METHODS

There were examined 153 men aged 40–70 years, including 53 patients with COPD, 56 with a combination of COPD and CHD and 44 patients with stable CHD. The control group consisted of 30 practically healthy individuals. The verification of the COPD diagnosis and severity of patients was based on the recommendations of WHO experts – GOLD (2018) [32]. The diagnosis of stable CHD was established in accordance with European recommendations (2013) [33].

The level of LP (a) was determined using enzyme-linked immunosorbent assay (ELISA) – Cormay reagent kit, Diagnostic Automation, Inc., Poland. The content of PAPP-A in serum was determined by enzyme immunoassay using IBL-INTERNATIONAL PAPP-A US (ultra sensitive) Enzyme Immunoassay Kit (Germany).

Statistical processing of the results was performed using the "Statistica" v.10.0 and «Microsoft Office Excel 2010». The reliability of the results was assessed using Student's t-test; discrepancies at $p<0,05$ were considered significant. The relationship of signs was determined using Pearson and Spearman correlation coefficients (r). The Mann-Whitney test was used to compare averages means in two independent groups.

## RESULTS

The level of LP (a) in the control group fluctuated in a significant range – from 6,78 to 20,4 mg/dL, averaging 14,37 ± 2,19 mg/dL. For the cut-off point from the standards, the LP (a) level was adopted – 15,50 mg/dL. The mean level of LP (a) was slightly elevated in 7 (13,20%) of 53 patients with COPD without CHD, averaged 18,53 ± 2,73 mg/dl ($p>0,05$). The increase in the level of LP (a) occurred in 23 (52,27%) of 44 patients with CHD, in the group with comorbid pathology in 34 of 56 patients – 60,71% ($p <0,05$). The average level of LP (a) was elevated both in the group of CHD patients (40,38 ± 1,84 mg/dl) and to a slightly greater extent in patients with cardiorespiratory pathology (46,55 ± 2,09 mg/dl) ($p <0,05$). Certain borderline LP (a) levels in the compared groups [34] were 15,91 mg/dl for the group of patients with isolated COPD, 27,96 mg/dl for patients with CHD and 30,56 mg/dl for patients with CHD and COPD.

The degree of change in the level of LP (a) did not depend on the severity of COPD, but was associated with the severity of CHD, both in monopathology CHD and in its combination with COPD. The level of LP (a) in patients with functional class III of angina pectoris was significantly higher than in patients with functional class II (respectively 43,56±1,88 versus 37,84±1,12 mg/dL in patients

with CHD and 48,65±2,14 versus 40,24±2,39 mg/dl in patients with a combination of COPD and CHD, p<0,05). An elevated level of LP (a) may increase the risk of cardiovascular disease due to the potentiation of atherogenesis as a result of the accumulation of LP (a) in the intima and prothrombotic effects of apoprotein Apo (A), which has a structural similarity to plasminogen molecule, but does not have fibrinolytic activity [35]. This circumstance allows us to consider the LP (a) as a marker of early and severe forms of CHD, independent of other risk factors, as indicated by other researchers [14,36]. The combination of elevated concentrations of LP (a) and other adverse risk factors (smoking, obesity, physical inactivity) or associated diseases, including COPD, further increases the risk of CHD [37]. It can be assumed that the presence of CHD in patients with COPD is decisive in the overproduction of LP (a) in comorbid pathology, and the combination of COPD + CHD is more unfavorable in relation to the development and severity of proatherogenic disorders of lipid metabolism, which may indicate the syndrome of mutual complication of diseases.

The level of PAPP-A in the control group was 3,12±0,42 mIU/L. In patients with stable CHD, the level of PAPP-A was moderate, but well above the control group (5,61±0,23 mIU/L, p<0,05). The degree of change in the level of PAPP-A in patients with COPD was not significant (4,03±0,32 mIU/L, p>0,05). The combination of CHD and COPD showed a slightly higher degree of increase in PAPP-A compared with the group of CHD patients without COPD (6,34±0,26 mIU/L, p<0,05). Boundary values of PAPP-A for the group of patients with COPD is 3,67 mIU/L, for patients with CHD – 4,56 mIU/L, for patients with a combination of COPD and CHD – 4,89 mIU/L. The sensitivity of PAPP-A determination for the diagnosis of CHD in patients with COPD is 79%, specificity – 72%.

There was a tendency to increase the level of PAPP-A with increasing functional class (FC) of stenocardia in patients with CHD without COPD: in patients with FC II, the content of PAPP-A was 4,98±0,28 mIU/L, and with FC III – 6,28±0,21 mIU/L (p<0,05). When combined CHD and COPD, the association of PAPP-A with severity of CHD was more pronounced: in patients with FC II, PAPP-A was 5,38±0,19 mIU/l; with FC III – 6,91±0,21 mIU/l (p<0,01).

## DISCUSSION

It can be assumed that the enhancement of local inflammation in the bronchi and pulmonary parenchyma has a systemic effect and contributes not only to the progression of COPD, but also to the activation of vascular inflammation, the development and progression of atherosclerosis, followed by destabilization of the atherosclerotic plaque [38,39]. This may be indicated by the presence of a correlation between the PAPP-A content and the level of C-reactive protein in the blood, determined by a highly sensitive method in patients with a combination of COPD and CHD (r=0,41; p<0,05). No convincing correlation was found between the level of PAPP-A with age (r=0,18), body mass index (r=0,17), LDL cholesterol level (r=0,20; p>0,05), blood pressure value (r=0,22; p>0,05). A moderate feedback was observed between the levels of PAPP-A and HDL cholesterol, which can be explained by the presence of the anti-inflammatory effect of HDL (r=-0,32; p<0,05). There is evidence that healthy people with HDL are characterized by the ability to inhibit the chemotaxis of monocytes, and their anti-inflammatory effect is 0.38, while people with CHD have a pro-inflammatory effect with an index equal to 1.38 [40]. An association has been established between the levels of LP (a) and C-reactive protein, which may indicate the potentiating effect of LP (a) on the process of nonspecific inflammation in the vascular wall in patients with CHD. Since the content of LP (a) in the blood is a genetically determined individual trait of the patient and, having reached stable values by 2 years, remains constant throughout life [41], its high level associated with the induction of inflammation can be considered a criterion of severe forms of CHD or it's exacerbations, including when combined with COPD.

## CONCLUSIONS

The study confirmed the importance of introducing into practice methods for determining new independent risk factors for the occurrence and progression of CHD in patients with COPD – levels of LP (a) and PAPP-A. An increase in LP (a) level more than 18 mg/dL can be considered in patients with COPD as a possible predictor of CHD, and its increase in excess of 30 mg/dL is a reliable criterion for CHD, indicating a hereditary predisposition to early development of coronary atherosclerosis. An elevated level of LP (a) is associated with more severe forms of CHD in patients with a stable course of CHD, both in the monopathology group and in combination with COPD.

Detection of elevated level of PAPP-A (more than 5 mIU/l) in the blood plasma of patients with COPD makes it possible to isolate risk groups for CHD even in the absence of its pronounced clinical manifestations. Increased PAPP-A levels in the blood plasma of CHD patients and with a combination of COPD and CHD are not associated with the generally accepted risk factors of CHD in patients, but correlate reliably with plasma concentration of CRP and LP(a) level, which may indicate an association of inflammation and destruction in the atherosclerotic process, its progression and the possible development of complications.

Determining the concentration of PAPP-A in the blood of patients with COPD may be an important laboratory marker of CHD. Increased plasma PAPP-A levels of more than 5 mIU/L in patients CHD and CHD in combination with COPD should be regarded as an indicator of damage an atherosclerotic plaque, a potential biomarker of its instability, and therefore, as a predictor of cardiovascular complications. The combination of COPD and CHD is characterized by more pronounced changes in the level of PAPPA-A and LP (a), which characterizes the mutual influence of these diseases.

There is a need for epidemiological studies to clarify the diagnostic and prognostic significance of PAPP-A and LP (a) in asymptomatic subjects and in subjects with documented CHD with cardiorespiratory comorbidity.

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ORIGINAL ARTICLE

# METABOLIC THERAPY IN THE COMPLEX TREATMENT OF CHRONIC PANCREATITIS WITH STABLE CORONARY ARTERY DISEASE

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## ABSTRACT

**The aim:** To study the effectiveness of using medicine meldonium in standard therapy to the correction of prooxidant-antioxidant and kallikrein-kinin disorders in patients with chronic pancreatitis andstable coronary artery disease.

**Materials and methods:** The study included 90 patients with chronic pancreatitis andstable coronary artery disease.They were divided into two groups: I group (45 patients) received standard treatment; II group (45 patients) along with basic therapy received medication meldonium (Vazonat) for 2 capsules (500 mg) once daily for two months. Indicators prooxidant-antioxidant system in blood plasma was determined by biochemical method, indicators of kallikrein-kinin system– by chromatographic method.

**Results:** The better status of the prooxidant-antioxidant system and kallikrein-kinin system was observed in patients who received in addition to standard protocol treatment with meldonium.

**Conclusions:** Adding to the complex therapy of patients with chronic pancreatitis and stable coronary artery disease of the medicine meldonium helps to improve the prooxidation-antioxidant status and disorders in the kallikrein-kinin system more significantly compared with standard basic therapy.

**KEY WORDS:** chronic pancreatitis, stable coronary artery disease, treatment, metabolic therapy, meldonium

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## INTRODUCTION

Chronic pancreatitis (CP) is one of the most common, rapidly progressing disease of pancreas with a high incidence of temporary disability and primary disability. It is known about the negative effect of CP on the cardiovascular system. It is established that in 15.5 % of patients with gastroenterological pathologies, including pancreatitis, there is stable coronary artery disease (SCAD). To date, the mechanisms for realizing the impact of the inflammatory process in the software on the development and progression of CP have not yet been fully understood [1]. The combination of CP and SCAD is characterized by a progressive course with increasing of the functional insufficiency of pancreas and the development of disorders in the kallikrein-kinin (KKS) system and prooxidant-antioxidant system [2, 3]. The uncertainty of these mechanisms leaves open the question of drug therapy for such a contingent of patients, which generally reduces the effectiveness of the treatment of patients with SCAD. Therefore, the search for effective treatment regimens in this direction is relevant for modern medicine [4, 5].

Standard basic therapy for comorbidity of CP and SCAD does not include the correction of prooxidant-antioxidant disorders and imbalances in the KKS [6, 7]. In recent years, the use of metabolic drugs has become common in medical practice. Meldonium, as a representative of metabolic therapy, refers to partial inhibitors of fatty acid oxidation. Its mechanism of action is related to the inverse limitation of the rate of biosynthesis of carnitine from its predecessor, gamma-butyrobetaine. As a result, carnitine-mediated transport of long-chain fatty acids through the mitochondrial membranes is inhibited without affecting the metabolism of short-chain fatty acids, thereby making meldonium capable of altering the prooxidant-antioxidant status and KKS [8].

The use of meldonium in combination with conventional therapy for CP combined with SCAD (nitrates, beta-blockers, angiotensin-converting enzyme inhibitors, statins, antiplatelet agents, antispasmodics, prokinetics, proton pump inhibitors, enzymes) allows to improve parameters of prooxidant-antioxidant status and imbalance in KKS.

Therefore, the use of meldonium in standard basic therapy for comorbidity of CP and SCAD is appropriate.

## THE AIM

To investigate the effectiveness of course treatment meldonium (Vazonat) in standard therapy for the correction of prooxidant-antioxidant and kallicrein-kinin disorders in patients with CP and SCAD.

## MATERIALS AND METHODS

The study was conducted at the Department of General Practice – Family Medicine, I. Horbachevsky Ternopil State Medical University (within 2016-2017).

To achieve this goal, 90 patients were selected from the CP in the stage of remission with MS. They were comparable to the etiological factor, socio-economic conditions and nutrition. Also, the influence of the alcohol factor was excluded. Among patients, there were 47 (52.22 %) male age (49.7±7.6) years and 43 female (47.78 %) age (51.15±6.4) years. The average duration of CP was (12.6±4.4) years, SCAD– (4.6±1.2) years. Patient examination was carried out with their consent. The study did not include patients with moderate to severe DM requiring insulin, severe arterial hypertension, myocardial infarction, cancer and somatic illness in the stage of decompensation. The studies meet the requirements of the Helsinki Declaration of the World Medical Association «Ethical principles for medical research involving human subjects as the object of study» opinion of the Committee on bioethics SHEI «Ternopil State Medical University by I. Horbachevsky of MPH of Ukraine»№ 41/2017.

Depending on the treatment program, the patients were divided into two groups: I group (45 patients)received standard protocol treatment (SPT) (creon 25.000 IU during meals, pantoprazole 40 mg once a day and/or domperidone 10 mg 3 times daily, atorvastatin 10 mg in the evening, in the presence of arterial hypertension – ramipril 5 mg in the morning, aspirin 75 mg in the evening, nebivolol 5 mg in the morning (with pulse control), nitrates as needed); II group (45 patients), in addition to SPT additionally received a medication of meldonium (Vazonat) for 2 capsules (500 mg) once daily for two months. The control group consisted of 20 practically healthy persons aged 19 to 46 years, the average age – (32.2±1.8) years. Among them there were 11 (55 %) men and 9 (45 %) women.

The diagnosis of CP was verified on the basis of the generally accepted classification in Ukraine proposed by the Scientific Research Institute of Medical Sciences of Ukraine, which corresponds to the Marseilles-Roman classification according to the «Unified clinical protocol of primary, secondary (specialized) medical care and medical rehabilitation of patients with chronic pancreatitis» approved by Order of the Ministry of Health of Ukraine № 638 dated 10.09.2014 [9]. The diagnosis of SCAD was established according to guidelines from the Health-National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA) [10, 11]. The state of prooxidant-antioxidant system was established by levels of malonic aldehyde (MA), superoxide dismutase (SOD), SH-groups, catalase and ceruloplasmin (CP) of blood, which were determined by biochemical methods. Indicators of KKS in blood plasma were determined by chromatographic method [12].Specific proteolysis was evaluated by the content of KK, PKK (prekallikrein), $\alpha_2$-MG ($\alpha_2$-macroglobulin), and kininase-II. Non-specific proteolysis was evaluated by the level of PAP (proteolytic activity of plasma) and $\alpha_1$-IP ($\alpha_1$-proteaseinhibitor) in blood plasma. All studied parameters were determined twice before and after treatment.

Statistical processing of the received data was performed on a personal computer using standard software packages of Microsoft Excel and with help of the computer program Statistica for Windowsversion6.0 (Stat Soft inc., USA).

## RESULTS

By analyzing the prooxidant-antioxidant indices before and after treatment in patients of the two study groups, we determined a statistically significant improvement in the entire spectrum of this system ($p<0.05$).In the study of the state of the indicators of the prooxidant-antioxidant system for the treatment of patients with CP and SCAD, it was found that the level of MA was significantly higher in the I and II group compared with the control and was accordingly (6.35±0.07) μmol/L and (6.39±0.09) μmol/L. After the treatment, the level of MA in I group significantly decreased by 1.40 μmol/l (22.05 %), whereas in II group, this indicator significantly decreased by 2.22 μmol/l (34.75 %) (Fig. 1).

Also, prior to treatment, there was a significant decrease in the activity of antioxidant system enzymes at the level of SOD (I group – (39.22±0.47) units, II group – (39.52±0.45) units) and SH-groups (I group – (38.55±0.47) mmol/l; II group – (38.52±0.45) mmol/L) in both groups compared to control. After the treatment, a more significant increase in

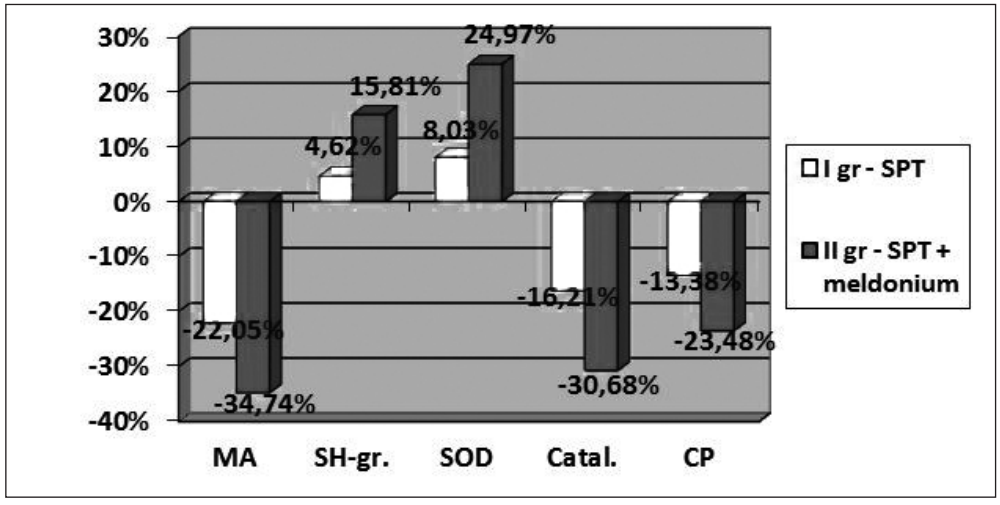


**Figure 1.** Dynamics of indicators of the prooxidant-antioxidant system after treatment in patients with CP and SCAD in comparison groups

**Table 1.** Dynamics of KKS under the influence of SPT with the in clusion of meldonium

| Indicator | Control group (n=20) | I group (n=45) | | II group (n=45) | |
|---|---|---|---|---|---|
| | | before treatment | after treatment | before treatment | after treatment |
| PAP, mmol / (h·L) | 31.83±0.71 | 55.68± 0.93* p2-3<0.05 | 49.03± 0.63# | 55.52± 0.76** p4-5<0,05 | 45.73± 0,48## p3-5<0.05 |
| KK, μmol / (min·L) | 52.15±1.43 | 177.51± 1.62* p2-3<0.05 | 164.16± 1.63# | 177.26± 1.59** p4-5<0.05 | 154.31± 0.84## p3-5<0.05 |
| PKK, μmol / (min·L) | 72.57±1.21 | 36.91± 0.67* p2-3<0.05 | 40.44± 0.73# | 36.94± 0.67** p4-5<0.05 | 43.08± 0.80## p3-5<0.05 |
| α1-IP, g/L | 1.41±0.02 | 1.98± 0.06* p2-3<0.05 | 1.82± 0.01# | 1.98± 0.02** p4-5<0.05 | 1.71± 0.01## p3-5<0.05 |
| α2-MG, g/L | 1.50±0.03 | 0,45±0,02* p2-3<0.05 | 0.56±0.01# | 0,45±0,02** p4-5<0,05 | 0,67±0,01## p3-5<0.05 |
| The activity of kininase-II, μmol / (min·L) | 269.84±1.74 | 152.97± 2.50* p2-3<0.05 | 165,44± 1.16# | 152.84± 2.00** p4-5<0.05 | 176.10± 2.12## p3-5<0.05 |

Note 1. * – (p1-2<0.05); Note 2. # – (p1-3<0.05);
Note 3. ** – (p1-4<0.05); Note 4. ## – (p1-5<0.05).

the activity of SOD (by 24.98 %) and an increase in the level of SH-groups (by 15.81 %) in II group was observed, while in the I group, these indices increased slightly and unreliable.

The level of catalase in blood plasma before treatment in I and II groups of patients was significantly higher compared to control ((55.72±1.12) % and (55.77±1.03) % respectively). After treatment, this indicator decreased significantly by 16.22 % in I group and 30.68 % in II group (Fig. 1).

Table 1 shows the results of the obtained indicators of general and specific proteolysis in the comparison groups that characterize the state of KKS before and after treatment.

While analyzing indicators of general and specific proteolysis, a positive effect of the treatment in I group and II group was observed, but in II group the therapeutic effect was more significant: the level of PAP decreased by 11.94 % in I group and by 17.63 % – in II group, the level of KK decreased by 7.52 % in I group and by 12.95% – in II group, the level of PKK increased by 9.56% in I group and by 16.62 % in II group. The levels of these indicators after treatment in two groups were statistically significantly higher than those before treatment (p<0.05). The level of $\alpha_1$-IP decreased significantly and statistically significantly in II group compared to I group by 6.04 % (p<0.05). With regarding to $\alpha_2$-MG and kininase-II levels, ther values in the SPT + meldonium group increased statistically significantly by 19.64% and 6.44 %, respectively relative to the SPT group (p <0.05).

## DISCUSSIONS

Assessing the state of the prooxidant-antioxidant system in patients with CP in combination with SCAD after treatment, we found a statistically significant decrease in the level of MA. From this we can conclude that the addition of meldonium to SPT led to a decrease in the intensity of lipoperoxidation processes. After treatment, there was also an increase in SOD and SH-groups in the two comparis on groups, but this in crease was more statistically significant in patients with CP in combination with SCAD who, in addition to SPT, received the drug meldonium. With regard to the CP level and the catalase in the blood of the patients under study, their levels after treatment alsodecreasedsignificantlymorestatistically inpatientswithadditionaluse of meldoniumin SPT. This demonstrates the regulatory ability of the metabolic agents in terms of antioxidant protection [13].

Therefore, the use of meldonium in the complex treatment of patients with CP in combination with SCAD leads to an improvement in antioxidant protection and to reduce the processes of lipidperoxidation [14].

Analyzing KKS rates in patients with CP with SCAD, we noted a more significant improvement in the group of patients with additional admission of meldoniumto SPT. Indicators of specific proteolysis after treatment were statistically significantly lower in patients of II group compared with patients of I group. From this we canconclude about the regulatory effect of meldonium on the imbalance in the specific proteolysis system. Regarding the in dices of nonspecific proteolysis, we observed the same positive effect of meldonium in patients of II group [15].

Therefore, on the basis of the above, it is advisable to use meldonium in SPT in patients with CP in combination with SCAD in the following scheme: 2 capsules (500 mg) once a day for 2 months to improve the prooxidant-antioxidant status and imbalance in KKS.

## CONCLUSIONS

1. Use in the complex treatment of patients with chronic pancreatitis and stable coronary artery disease medication of meldonium contributed to a more reliable regression of prooxidant-antioxidant disorders in comparison with standard conventional therapy($p<0.05$).
2. Adding to the basic therapy of patients with chronic pancreatitis and stable coronary artery disease medication of meldoniumled to a more significant improvement in balance of kallikrein-kinin system ($p<0.05$) than with standard protocol treatment.

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ORIGINAL ARTICLE

# THE EFFECTS OF L-ORNITINE AND L-ARGININE ON THE PROCESSES OF LIPID PEROXIDATION IN THE FUNCTIONAL LAYERS OF KIDNEYS ON THE BACKGROUND OF ACUTE TOXIC HEPATITIS

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## ABSTRACT

**The aim** is to evaluate the effects of L-arginine and L-ornithine on the processes of lipid peroxidation in homogenates of renal cortex, renal medulla and renal papilla under conditions of acute toxic hepatitis.

**Materials and methods:** The study was performed on 40 outbred white male rats with experimental hepatitis, caused by carbon tetrachloride. The animals were divided into five groups: control group (the rats were simulated carbon tetrachloride poisoning and its correction by administering of olive oil and normal saline in equivalent doses), acute carbon tetrachloride hepatitis (single intraperitoneal injection of 50% carbon tetrachloride oil solution at the dose of 2 mlxkg$^{-1}$ of body weight and simulation of treatment by administration of normal saline in equivalent doses), acute carbon tetrachloride hepatitis + L-ornithine (1000 mgxkg-1), acute carbon tetrachloride hepatitis + L-arginine (500 mgxkg-1) and acute carbon tetrachloride hepatitis + combination of substances.

**Results:** On the background of acute carbon tetrachloride intoxication it was observed the development of renal failure in experimental animals, manifested by activation of lipid peroxidation processes in homogenates of renal cortex, renal medulla and renal papilla. The administration of L-ornithine and L-arginine demonstrates positive impact on renal function and hepato-renal syndrome by stabilization of cell membranes and regeneration of functional capacity of injured renal cells.

**Conclusions:** The results of our study confirm both the presence of unidirectional effects and absence of toxic influences of L-ornithine and L-arginine on renal cells under the conditions of acute carbon tetrachloride intoxication, which are the most important requirements for modern drugs for the treatment of hepato-renal syndrome.

**KEY WORDS:** carbon tetrachloride, liver, kidney, hepato-renal syndrome, L-ornithine, L-arginine

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## INTRODUCTION

The disorders of hepatobiliary system occupy a significant place in the clinic of internal diseases. The liver, as an organ that plays a leading role in the regulation of metabolism, body integrity, homeostasis support, xenobiotic neutralization, is the target organ of foreign compounds, a significant percentage of which have selective hepatotoxicity. Among them, industrial poisons and medicines take one of the significant places. According to modern data, in Ukraine, 70-80% of chronic liver diseases occurs as a result of previous viral hepatitis, 20-30% — develops due to toxic injuries [1]. Impaired elimination of toxic metabolites, associated with renal parenchyma damage, leads to their retention in the organism and worsening of intoxication, which contributes to the development of acute or chronic hepatorenal insufficiency. Clinical manifestations of combined toxic liver and kidney damage occur in about 30% of acute toxicities. In a study of patients with alcoholic hepatitis, for example, hepatorenal syndrome occurred in 28 of 101 patients [2].

A promising direction for the correction of liver toxicity is the use of drugs, containing natural amino acids, which, entering into metabolism, have a positive impact on hepatic functional recovery. The effectiveness of L-ornithine and L-arginine administration in liver pathology has been shown in a number of papers [3, 4]. However, the disruption of lipid peroxidation processes in the renal tissues on the background of carbon tetrachloride intoxication has not been sufficiently studied; there is no data about the effect of combined use of above-mentioned biologically active substances in order to inhibit activated peroxidation reactions, triggered by compounds with high toxicity.

## THE AIM

The aim of the study is to evaluate the effect of L-arginine and L-ornithine on the processes of lipid peroxidation (POL) in functional layers of the kidney under conditions of acute intoxication with carbon tetrachloride.

## MATERIALS AND METHODS

The study of the therapeutic effect of amino acids L-ornithine and L-arginine on the processes of POL in

**Table 1.** Combined effect of L-ornithine and L-arginine on the state of POL in homogenates of renal cortex, renal medulla and renal papilla under the conditions of acute toxic hepatitis (M±m)

| Index | Control group (n=8) | Hepatitis (n=5) | Hepatitis + L-ornithine (n=7) | Hepatitis + L-arginine (n=6) | Hepatitis + combination (n=8) |
|---|---|---|---|---|---|
| DC (renal cortex), au×g$^{-1}$ | 0,448± 0,011 | 0,745± 0,060$^{\#\#\#}$ | 0,655± 0,039$^{\#\#\#}$ | 0,555± 0,035$^{\#*}$ $p_1$>0,05 | 0,507± 0,036$^{**}$ $p_1$<0,05 $p_2$>0,05 |
| DC (renal medulla), au×g$^{-1}$ | 0,421± 0,010 | 0,930± 0,058$^{\#\#\#}$ | 0,700± 0,050$^{\#\#\#*}$ | 0,605± 0,042$^{\#\#**}$ $p_1$>0,05 | 0,458± 0,037$^{***}$ $p_1$<0,01 $p_2$<0,05 |
| DC (renal papilla), au×g$^{-1}$ | 0,443± 0,007 | 0,815± 0,032$^{\#\#\#}$ | 0,752± 0,053$^{\#\#\#}$ | 0,757± 0,040$^{\#\#\#}$ $p_1$>0,05 | 0,600± 0,044$^{\#\#**}$ $p_1$<0,05 $p_2$<0,05 |
| TBA-reactive substances (renal cortex), mcmol×kg$^{-1}$ | 1,033± 0,029 | 2,013± 0,094$^{\#\#\#}$ | 1,252± 0,077$^{\#***}$ | 1,668± 0,121$^{\#\#\#}$ $p_1$<0,05 | 1,388± 0,080$^{\#\#\#***}$ $p_1$>0,05 $p_2$>0,05 |
| TBA-reactive substances (renal medulla), mcmol×kg$^{-1}$ | 1,123± 0,022 | 1,850± 0,106$^{\#\#\#}$ | 1,303± 0,093$^{**}$ | 1,730± 0,119$^{\#\#\#}$ $p_1$<0,05 | 1,550± 0,065$^{\#\#\#*}$ $p_1$<0,05 $p_2$>0,05 |
| TBA-reactive substances (renal papilla), mcmol×kg$^{-1}$ | 1,065± 0,037 | 1,930± 0,070$^{\#\#\#}$ | 1,192± 0,065$^{***}$ | 1,875± 0,111$^{\#\#\#}$ $p_1$<0,001 | 1,352± 0,104$^{\#}$ $p_1$>0,05 $p_2$<0,01 |

Notes:

1. # – statistical significance of indexes compared with control group (# – p<0,05; ## – p<0,01; ### – p<0,001);
2. * – statistical significance of indexes compared with group of animals with hepatitis (* – p<0,05; ** – p<0,01; *** – p<0,001);
3. p1 – statistical significance of indexes compared with group of animals with hepatitis, treated with L-ornithine;
4. p2 – statistical significance of indexes compared with group of animals with hepatitis, treated with L-arginine.

kidney tissues under the conditions of toxic hepatitis was performed on 40 outbred white male rats, with the weight of 180-220 g, which were kept under vivarium conditions on a standard diet. All manipulations on the animals were carried out in accordance with the provisions of the "European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes" (ETS No. 123, Strasbourg, 18.03.1986), "Guide for the Care and Use of Laboratory Animals" (National Academies Press, USA, 2011), in compliance with the Law of Ukraine "On the Protection of Animals from Cruelty" No. 27, Art. 230, from 2006, with the changes, made in accordance with the Law No. 1759-VI (1759-17) from December 15, 2009. Ethics Commission of medical and biological researches, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Protocol No. 57 from March 25, 2020) did not find any violations of moral and ethical standards during experiments on animals.

Animals were divided into 5 groups: the first group consisted of control animals, that were simulated of carbon tetrachloride poisoning and therapeutic measures by intraperitoneal administration of olive oil and normal saline in equivalent doses; the second group included rats, that were modeled of acute toxic hepatitis by a single intraperitoneal injection of 50% oil solution of carbon tetrachloride at a dose of 2 ml×kg$^{-1}$ of body weight of the animal [5] and simulated therapeutic effects by administration of normal saline in equivalent doses; in the third group, 1 day after modeling of hepatitis, the animals received intraperitoneal treatment with L-ornithine solution at a dose of 1000 mg×kg$^{-1}$ during seven days [6]; in the fourth group — with L-arginine solution at a dose of 500 mg×kg$^{-1}$ [7]; in the fifth group both substances were combined.

On the eighth day after the beginning of the corrective measures, all experimental animals were euthanized by the method of total bloodletting from the heart, and biochemical tests of the kidney homogenate were performed: the levels of lipid peroxides (TBA-reactive substances and diene conjugates (DC) [8] in renal cortex, renal medulla and renal papilla were determined. Statistical analysis of the results was performed using Student's t-test.

## RESULTS

Acute intoxication with carbon tetrachloride led to the activation of POL in the renal tissues (Table 1), which was manifested by a marked increasing of DC level. Thus, after the simulation of toxic hepatitis, the level of this marker was 1.66 times ($p<0.001$) higher in renal cortex, 2.20 times ($p<0.001$) higher in renal medulla, and 1.84 times ($p<0.001$) higher in renal papilla as compared to control group. After a seven-day correction with L-ornithine, no significant difference was observed between the levels of DC in renal cortex and renal papilla, comparing to the animals without any correction. But, at the same time, the level of DC in renal medulla decreased by 32.85% ($p<0.05$), while remaining at 66.27% higher than in control group ($p<0.001$).

As a result of therapeutic use of L-arginine, the level of DC in renal cortex decreased by 25.50% ($p<0.05$), compared to the animals without correction, but remained 23.88% ($p<0.05$) higher than in control group of animals. In the renal medulla, this index decreased by 65.05% ($p<0.01$) and exceeded the control by 43.70% ($p<0.01$).

After seven days of administration of the combination of amino acids, the level of DC in renal cortex was 68.05% ($p<0.01$) lower than in animals without correction and was not statistically significantly different, than in control group ($p>0.05$). This result was better than with the isolated administration of L-ornithine (the level of DC decreased by 22.59%, $p_1<0.05$), but it wasn't statistically significant different after administration of L-arginine ($p_2>0.05$). Under the influence of combined correction, the level of DC in renal medulla decreased by 2.03 times relative to the group of animals without correction, which was 34.57% better than when using L-ornithine ($p_1<0.01$) and 24.29% better than using L-arginine ($p_2<0.01$). Under these conditions, the following changes in the processes of POL were observed in the renal papilla after combination therapy: the level of DC decreased by 73.62% relative to the group of animals without correction ($p<0.01$) and remained 35.44% higher than in the control group of rats ($p<0.01$). This index was 20.21% and 20.73% lower, respectively, then with isolated administration of L-ornithine ($p_1<0.05$) and L-arginine ($p_2<0.05$).

On the 8th day after administration of carbon tetrachloride, significant increasing of the levels of TBA-reactive substances was observed in renal cortex, renal medulla and renal papilla (Table 1). Comparing to the control group, this index increased by 94.86%, 60.70% and 81.22% ($p<0,001$), respectively, indicating a pronounced nephrotoxic effect of this compound.

Under the influence of corrective treatment on the investigated pathological process with L-ornithine, the following changes were observed in renal cortex: the level of TBA-reactive substances decreased by 62.19% ($p<0.001$) compared with animals without correction, which remained 17.49% ($p<0.05$) higher than in control group. In renal medulla this index decreased by 41.98% ($p<0.01$) and reached the level of control group after the treatment with L-ornithine. According to the content of TBA-reactive substances in renal papilla, the following should be noted: under the influence of L-ornithine this index decreased by 61.91% ($p<0.001$) comparing to the group of animals without correction, which was not statistically significantly different from the control level ($p> 0.05$).

Therapeutic use of L-arginine did not lead to significant changes in the content of TBA-reactive substances relative to the group of animals without correction. Comparing with the control group of animals, in renal cortex this index exceeded the control level by 61.47% ($p<0.001$), in renal medulla — by 54.05% ($p<0.001$), and in renal papilla — by 76.05% ($p<0.001$). The results, obtained in renal cortex and renal medulla, were 33.22 % and 32.77% lower, respectively, than after the administration of L-ornithine ($p_1<0.05$), and 63.57% higher than in the renal papilla ($p_1<0.001$).

The combined use of these amino acids with therapeutic purposes in rats with acute toxic hepatitis contributed to the following changes in the content of TBA-reactive substances in the kidney tissues: in renal cortex this index decreased by 68.95% ($p<0.001$) relative to animals without correction, in renal medulla — by 70.05% ($p<0.05$), and in renal papilla the differences were not statistically significant ($p>0.05$). At the same time, the concentration of TBA-reactive substances in renal cortex remained 34.36%, ($p<0.001$) higher, comparing to the control group, in renal medulla — 38.02% ($p<0.001$), and in renal papilla — 26.94% ($p<0.05$) higher, respectively. Comparison of data in all experimental groups with each other showed that after the combine use of investigated amino acids, the content of TBA-reactive substances in renal cortex was not significantly different from that, obtained after individual administration L-ornithine and L-arginine. In renal medulla this index became statistically significantly higher, than with the introduction of L-ornithine (18.95%, $p_1<0.05$), and in renal papilla — 72.10% lower, than after administration of L-arginine ($p_2<0.01$).

## DISCUSSION

As can be seen from the above, the results of our investigation showed that the modeling of acute toxic hepatitis is accompanied by synchronous injury of renal parenchyma, which is manifested by the development of hepato-renal syndrome with a significant increase in the content of primary and secondary products of LPO in homogenates of renal cortex, renal medulla and renal papilla. The obtained data are consistent with the results of studies of other authors [9, 10, 11]. The use of L-ornithine and L-arginine for the corrective purposes is accompanied by a pronounced positive effect. Moreover, it should be noted, that evaluating the changes of DC content, it was observed, that therapeutic potency was more significant after combined use of investigated amino acids, while L-ornithine administration was more effective relative to the content of secondary products of LPO. According to present knowledge, L-ornithine has the ability to enhance the regeneration processes in the affected cells of the body by stimulation the synthesis of spermine and spermidine. However, many scientists believe that this effect is possible

only with the normalization of liver hemodynamics [12, 13], which in this case is obviously related to the stimulation of nitric oxide (NO) synthesis in endothelial cells. The physiological role of NO is the relaxation of the smooth muscle of the blood vessels, which leads to improvement of microcirculation and hepatic circulation. The link between the ornithine cycle and the nitric oxide cycle is the amino acid L-arginine, which is a direct precursor of L-ornithine and a substrate for NO-synthase, an enzyme that catalyzes the synthesis of nitric oxide. As a result, it can be assumed that this complex of amino acid substances contributes to the formation of the required pool of L-arginine, as a precursor of NO, which under experimental conditions obviously contributes to the improvement of microcirculation, leading to a faster restoration of cell integrity and functional capacity of liver cells. As a lipophilic molecule, NO easily diffuses through cell membranes into the neighboring cells (e.g. from endothelial to monocytes of vessels), where cyclic guanozine monophosphate decreases the concentration of free calcium and activates myosin light chain kinase causing vessel dilatation [14, 15]. Recent study demonstrated that L-arginine supplementation in type II diabetic rats was beneficial by preserving glomerular filtration rates, presumably via increased renal endothelial nitric oxide synthase levels, that leads to renal vasodilation [16]. At the same time, L-arginine in the standard therapy facilitates the correction of lipid peroxidation processes and reduces the severity of microalbuminuria [17].

The beneficial effect of L-ornithine is also described in a number of trials in patients with hepatic encephalopathy. A 2017 randomized clinical trial compared the effect of intravenous L-ornithine versus placebo in reverting over overt hepatic encephalopathy (OHE) at day 5 of treatment in a total of 193 patients with cirrhosis. The authors conclude that L-ornithine shortens the recovery time from OHE and the duration of hospitalization [18]. A meta-analysis of L-ornithine versus placebo or other interventions (lactulose, probiotics, and/or rifaximin) included 26 randomized clinical trials involving 1783 patients [19]. L-ornithine had a beneficial effect on hepatic encephalopathy (RR 0.60, 95% CI 0.44–0.82) and was associated with reduced mortality (RR 0.42, 95% CI 0.22–0.84).

Consequently, it may be summarized that the amino acids L-arginine and L-ornithine act as synergists, potentiate each other, and their combined administration leads to the normalization of the structural and functional capacity of the liver and kidney, affected by carbon tetrachloride.

## CONCLUSIONS

1. First of all, it should be noted, that the amino acids L-arginine and L-ornithine are able to alleviate the pathogenic mechanisms of toxic effects of carbon tetrachloride on the liver and kidneys.
2. These investigated substances, both individually and in combination, have the ability to reduce the activity of lipid peroxidation processes in the functional layers of the kidneys, which have a beneficial effect on the stabilization of cell membrane and the restoration of functional capacity of the affected kidney cells.
3. This indicates the absence of toxic effects of L-arginine and L-ornithine on nephrocytes, which is one of the most important requirements for modern drugs for the treatment of hepato-renal syndrome.

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ORIGINAL ARTICLE

# INFLUENCE OF SYSTEMATIC TAKING BLOCKERS OF H2-HISTAMINE RECEPTORS ON THE DEGREE OF SEMINATION OF GASTRIC MUCOSA WITH *HELICOBACTER PYLORI* INFECTION OF PATIENT WITH CHRONIC NON – ATROPHIC GASTRITIS

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## ABSTRACT

**The aim:** To determine the effect of prolonged use of $H_2$-histamine receptor blockers on the degree of contamination of the gastric mucosa with HP infection in patients with chronic non-atrophic gastritis.

**Materials and methods**: 28 patients with chronic atrophic gastritis (the main group), who regularly took H2-histamine receptor blockers for 2 to 7 years, and 30 patients (control group), who never used them were comprehensively examined. Comprehensive examination included: step-by-step intragastric pH-metry, esophagogastroduodenoscopy, helicobacter infection test (HP) (helicobacter urease test and microscopic examination of stained smears), histological investigations of the gastric stump mucous, material for which was taken during endoscopy from 4 topographical zones: from the middle third of the gastric antrum and body of stomach on the big and small curvature.

**Results**: All the patients in 100% of cases have confirmed the existence of chronic non-atrophic gastritis in both active and inactive stages of varying degrees of severity. Helicobacter infection was detected in 100% of cases. A comparative analysis of the data on the average degree of infection of the gastric mucosa by HP infection in the same topographic zones in the patients of the main and control groups revealed a significant ($p < 0.05$) higher degree of seeding of the gastric mucosa in patients of the main group in all zones.

**Conclusions:** Monotherapy for chronic non-atrophic gastritis with blockers of $H_2$-histamine receptors leads to an increase in the degree of gastric mucosa semination with HP infection. This fact requires mandatory parallel use of antibacterial agents – colloidal bismuth subcitrate and antibiotics, with blockers of $H_2$-histamine receptors.

**KEY WORDS:** chronic non-atrophic gastritis, Helicobacter infection, blockers of $H_2$-histamine receptors

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## INTRODUCTION

The discovery of *Helicobacter pylori* (HP) in the 1983 year by Australian scientists B. Marshall and J. R. Warren changed views on etiology of such diseases as chronic gastritis type B (chronic non-atrophic gastritis), peptic ulcer disease, stomach cancer and MALT-Lymphoma [1, 2, 3, 4, 5, 6]. Before this discovery, it was thought that peptic ulcer disease is formed as a result of damage to the gastric mucosa and duodenal ulcer acid-peptic factor that led to the creation of different groups of drugs that affect the function of parietal cell acid – $M_1$-cholinolitik, blockers of $H_2$-histamine receptors and Proton pump inhibitors (PPIs). And although the Maastricht consensus all convocations (last 5-th the Maastricht consensus was adopted in the year 2015 (Florence) for the treatment of chronic *Helicobacter pylori* infection it is recommended to use quadratherapy, which, in addition to PPI and 2 antibiotics, includes preparation of the bismuth colloidal subcitrate [7, 8] widespread use will still remain the blockers of $H_2$-histamine receptors [9, 10, 11, 12, 13]. In the available for us literature there's no information on the impact of the drugs on the degree of gastric mucosa semination with HP infection that led to conduction of our research.

## THE AIM

To determine the effect of prolonged use of $H_2$-histamine receptor blockers on the degree of contamination of the gastric mucosa with HP infection in patients with chronic non-atrophic gastritis.

## MATERIALS AND METHODS

On the basis of the clinical division of the basic research laboratory on issues related to chronic *Helicobacter pylori* infection of Petro Mohyla Black Sea National University was comprehensively surveyed 28 patients with chronic non atrophic gastritis (main group), who regularly took the blockers of $H_2$ – histamine receptors within from 2 to 7 years, and 30 patients (control group) who had never taken them. The age of patients ranged from 25 to 58-years (the medium age was 33.2 ± 1.23); males were 37 (53.5%), females -21 (46.5%).

The study was conducted in accordance with the basic bioethical provisions of the Helsinki Declaration of the World Medical Association on the ethical principles of scientific 549 medical research involving human (2013) and the order of the Ministry of Health of Ukraine No.

**Table I.** The acidity level in the patients of the main and control groups

| Level of acidity | Main group (n=28) | | Control group (n=30) | |
|---|---|---|---|---|
| | Quantity of patients | % | Quantity of patients | % |
| Hyperacidity expressed | 1 | 3,6 | 1 | 3,3 |
| Hyperacidity moderate | 3 | 10,7 | 2 | 6,7 |
| Normacidity | 10 | 35,7 | 12 | 40 |
| Hypoacidity moderate | 7 | 25 | 8 | 26,7 |
| Hypoacidity expressed | 7 | 25 | 7 | 23,3 |
| Anacidity | 0 | 0 | 0 | 0 |

Note: n-the number of studies

**Table II.** Degree of semination of gastric mucosa with HP infection on topographical zones of patients with chronic non-atrophic gastritis of the main and control groups

| Groups | Degree of semination of gastric mucosa with HP infection on topographical zones of stomach ( + ) / (M ± м) | |
|---|---|---|
| | Antrum | Corpus of stomach |
| Main group (n = 28) | a) 2,95 ± 0,28; b) 2,98 ± 0,28. | a) 2,91 ± 0,28; b) 2,98 ± 0,28. |
| Control group (n = 30) | a) 2,05 ± 0,25; b) 1,97 ± 0,25. | a) 2,13 ± 0,25; b) 2,21 ± 0,25. |

Note: n-the number of studies, a)-large curvature, b)-small curvature.

690 dated September 23, 2009, which was confirmed by the findings of the meeting of the Ethical Commission of Petro Mohyla Black Sea National University, Nikolaev No. 12 dated December 11, 2019. A written consent was obtained from the patients for the study.

Primary comprehensive survey included: step-by-step intragastric pH-metry based on methodology by Chernobrovyi V.N esophagogastroduodenoscopy (EGDS) by generally accepted method, double HP testing: test for ureaz activity and microscoping stained by Giemsa smears, material for which was taken during endoscopy from 4 topographical zones: from the middle third of the gastric antrum and body of stomach on the big and small curvature with our created methodology and also histological studies of the gastric mucosa, the material for which is taken from the same zone, using a generally accepted method taking into account recent classification [14, 15].

Primary sequence survey: first patients conducted pH-metry and then EGDS with biopsy material for testing in HP and histological studies of the stomach mucosa. The study was conducted in the morning on an empty stomach, in 12-14 hours after the last meal. The data obtained were processed statistically using Student t-test with the computation of averages (m) and perhaps the likelihood of deviations (m). The changes were considered to be statistically significant at p 0.05 <. Statistical calculations were performed using Excel tables for Microsoft Office.

## RESULTS AND DISCUSSION

Data obtained during the conduct of pH-metering are shown in table I.

While conducting esophagogastroduodenoscopy active ulcerative process was not identified as in the stomach so as in duodenum, but 5 (8.6%) patients had manifestations coming through in the past ulcers duodenal bulb as scar deformity of varying degrees of severity. When analyzing data of histological investigations all the patients in 100% of cases have confirmed the existence of chronic non-atrophic gastritis in both active and inactive stages of varying degrees of severity.

When testing on HP Helicobacter infection was detected in 100% of cases. Data on the extent of the gastric mucosa for semination of HP infection on topographical zones of the stomach of patients with chronic non-atrophic gastritis are shown in table II.

Comparative analysis of data on the medium degree of semination with HP infection on gastric mucosa on similar topographical areas main group patients and control groups was detected significantly ($p < 0.05$) greater degree of semination of gastric mucosa of patients of basic group in all areas.

These results are understandable from the viewpoint of influence $H_2$ -histamine receptors on parietal cell (PC) and patterns of interactions of macroorganism – organism of a human being and microorganism.

As it is well known, there are three receptors on the PC through which goes the regulation developing of hydrochloric acid: histamine, gastrin, and acetylcholine. In contrast to the PPI, which inhibit the production of hydrochloric acid by blocking the $H^+/K^+$-ATPhase and without affecting the PC, blockers of $H_2$-histamine receptors affect only on the histamine receptors, which leads to a decrease in the formulation of hydrochloric acid at 70% in contrast to PPI, which block the production of nearly 100% [16]. Interaction between human body and HP infection is based on the impact of the protective properties of the organism on HP and means to combat bacteria to these protective properties. Protective factors of the human body is the effect of immune system and hydrochloric acid on HP infection.

However, HP has its own protective mechanisms: the bacterium produces a large number of factors of antioxidant system-superoxide dismutase and catalase, which neutralize the antibacterial activity of neutrophils and hydrochloric acid is neutralizes due to ammonia, which is produced from food under the influence of urea enzyme urease produced by HP [1]. Combating the factors of protection takes about 2/3 of energy reserves bacteria, while maintaining vital functions including reproduction-1/3 [1]. With incomplete reducing production of hydrochloric acid under the influence of $H_2$-histamine receptors energy costs to combat it is declining, and the bacterium gets more energy for reproduction, that is confirmed by the results of our research.

## CONCLUSIONS

1. Monotherapy for chronic non-atrophic gastritis with blockers of $H_2$-histamine receptors leads to an increase in the degree of gastric mucosa semination with HP infection.
2. This fact requires mandatory parallel use of antibacterial agents – colloidal bismuth subcitrate and antibiotics, with blockers of $H_2$-histamine receptors.

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ORIGINAL ARTICLE

# THE METHOD OF FORECASTING AS AN IMPORTANT STAGE IN SOLVING THE PROBLEMS FACING HEALTH IN THE FIELD OF MEDICAL CARE OF THE POPULATION

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## ABSTRACT

**The aim:** Of the work is to investigate and prove the effectiveness of medicinal products of plant origin for the treatment of cardiovascular diseases.

**Materials and methods:** Pharmacoeconomic, social, medical demographic and statistical studies were studied. The study involved cardiologists, pharmacists, consumers of pharmacies and patients of cardiology departments.

**Results**: Forecasting the need for cardiac medicines in general, and in medicinal products of plant origin (MP PO), in particular, is increasing as the population, life expectancy, under the influence of socio-demographic factors, and, most importantly, the increase in the number of elderly people. When solving problems facing public health in the field of drug provision of the population, a regular analysis of the pharmaceutical market and a forecast of the need for medicines in the context of the pharmacotherapeutic group that is being studied are necessary. An important stage in the choice of forecasting methods is the possibility of attracting the required number of indicators, which is a characteristic of the normative method, whose accessibility and simplicity make it possible to use it by specialists who do not have special mathematical training at the level of medical and pharmaceutical organizations.

**Conclusions**: Prevention of diseases should be based on the implementation of government programs for prevention and promotion of health. This will improve and actively use methods of early detection of social and economic factors, which is a threat of development of diseases of the cardiovascular system.

**KEY WORDS:** medicinal products of plant origin, cardiovascular diseases, marketing research, demand forecasting

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## INTRODUCTION

At the stages of marketing research, the study of the assortment, the solvency of consumers should be conducted with the obligatory consideration of regional peculiarities, the real financial possibilities of the medical and preventive institution and the solvency of the population, as well as factors influencing the choice of the medicinal product by medical, pharmaceutical workers and the population [1, 2].

In the pharmaceutical market of Ukraine, MP PO dominated by drugs domestically, which significantly reduces the cost of therapy and makes many drugs affordable for the socially unprotected strata of the population [3].

A characteristic feature of the task of determining the need for highly effective medicinal products of plant origin is the presence of unpredictable factors affecting the complex system of formation and satisfaction of demand (the emergence of new medicines, different levels of doctors' awareness, educational work among the population, etc.) [4].

Taking into account the peculiarities of the current social and economic situation, the inadequacy of financial drug assistance at the expense of the state budget and the need to address the problems of improving the organization of medicinal care for patients with cardiovascular pathology, we have forecasted the long-term demand for medicinal products of plant origin.

## THE AIM

The aim of the work is to investigate and prove the effectiveness of medicinal products of plant origin for the treatment of cardiovascular diseases.

## MATERIALS AND METHODS

Pharmacoeconomic, social, medical demographic and statistical studies were studied. The study involved cardiologists, pharmacists, consumers of pharmacies and patients of cardiology departments.

## RESULTS

The problem of reliability of providing consumers of medicinal products of plant origin directly depends on the creation of

**Table I.** Analysis of the frequency of medicinal products of plant origin for the treatment of cardiovascular disease (2006-2016)

| Name of medicinal products of plant origin | The average price of the package (S) | The number of appointments | Number of packages for the course of treatment (X) | n | к |
|---|---|---|---|---|---|
| Digoxin tablets 0,25 mg № 30 | 8-40 | 323 | 1 | 156 | 0,37 |
| Digoxin, solution for injection 0,25 % - 1ml №10 | 28-20 | 20 | 1 | 71 | 0,17 |
| Corglycon, solution for injection 0,06% - 1 ml № 10 | 21-20 | 335 | 1 | 53 | 0,13 |
| Platyphyllini hydrotartras 0,2 % - 1 ml № 10 | 28-50 | 500 | 1 | 40 | 0,48 |
| Papaverine hydrochloride 2 % - 2 ml № 10 | 20-20 | 1303 | 1 | 200 | 0,22 |
| Strophantine D | 22-20 | 200 | 1 | 32 | 0,08 |
| Strophantine | 21-20 | 10 | 1 | 15 | 0,04 |

an optimal stock of these medicines. WHO recommends the following approach: «The goal is to ensure that the size of the supply meets the demand, while the stock of medicines should be minimal, but to ensure that they are available in the event of an unexpected delay in delivery» [5].

An important stage in the choice of forecasting methods is the possibility of attracting the required number of indicators, which is a characteristic of the normative method, whose accessibility and simplicity make it possible to use it by specialists who do not have special mathematical training at the level of medical and pharmaceutical organizations.

The basis of this method is calculated norms of consumption of drugs, which reflect the characteristic properties and regularities of the normalized object. Calculation of the rate of consumption of drugs is based on the study of morbidity by nosological forms and the optimal set of drugs for the course of treatment, as well as the quantitative consumption of these drugs while being prescribed to the patient.

The advantage of this method is the ability to take into account changes in factors that affect the need. The most difficult problem of using the normative method is the considerable laboriousness of the development of norms, which requires the processing and analysis of large amounts of information.

The application of the normative method requires the calculation of the following indicators:
- forecast of the number of medicinal products of plant origin users;
- consumption intensity factors for each drug;
- consumption of each medicinal product for one year of use by one consumer.

Calculation of the need for drugs for the treatment of i-th nosology in the hospital in physical terms (in packages) for both outpatient and inpatient patients was carried out according to the formula:

$$Nij = Xij\ fact * Iij * Ei \quad (1),$$

where: Nij – the need for the j-th drug in the i-th nosology;

i – disease nosology;

j – specific drug;

Xij – an approximate norm of the need of j-drug for the course of treatment of one patient;

Iij – intensity factor of the j-drug requirement;

Ei – expected number of patients in the i-clinical group.

According to the proposed methodology, we determined the long-term need in 2011-2017 years in medicinal products of plant origin. The consumption of this group of drugs is predictable, since most drugs are prescription drugs. This fact makes it possible to accurately take into account the number of consumers in polyclinics and hospital departments. The need is calculated separately for each drug name in both quantitative and summary terms. In sum, the need for a drug for a course of treatment for one patient was determined by the formula

$$S = Nij * C \quad (2),$$

where: Nij – the need for medicinal product in quantitative terms;

C – the cost of medicinal product.

According to the scheme for determining the need for medicinal products of plant origin, it is necessary to know:
- the optimal assortment of medicinal products of plant origin;
- the amount of medicinal products of plant origin for one patient;
- the intensity of consumption of the medicinal product;
- the planned number of patients.

At the first stage, the intensity of consumption of drugs was calculated.

Calculation of the intensity of consumption, which was conducted on the basis of the study of letters of appointment in the history of the disease of cardiac patients of the cardiac dispensary. The intensity factor of consumption shows how often each specific medication is prescribed, in how many percentages of cases it is used, which part of consumers in total use this medication, and is calculated by the formula:

**Table II.** Calculation of hospital needs in medicinal products of plant origin which used to treat patients with cardiovascular pathology

| Name of medicinal products of plant origin | Forecast needs for years | | | | | |
|---|---|---|---|---|---|---|
| | 2015 | | 2016 | | 2017 | |
| | absolute unit | sum | absolute unit | sum | absolute unit | sum |
| Digoxin tablets 0,25 mg № 30 | 333 | 2797-20 | 343 | 2881-20 | 353 | 2965-20 |
| Digoxin, solution for injection 0,25 % - 1 ml № 10 | 21 | 592-20 | 22 | 620-40 | 23 | 648-60 |
| Corglycon, solution for injection 0,06 % - 1 ml № 10 | 345 | 7314-00 | 355 | 7526-00 | 366 | 7759-20 |
| Platyphyllini hydrotartras 0,2 % - 1 ml № 10 | 515 | 14677-50 | 530 | 15105-00 | 546 | 15561-00 |
| Papaverine hydrochloride 2 % - 2 ml № 10 | 1342 | 26840-00 | 1382 | 27640-00 | 1423 | 28460-00 |
| Strophantine D | 2060 | 45732-00 | 2122 | 47108-40 | 2186 | 48529-20 |
| Strophantine | 21 | 233-20 | 12 | 254-40 | 13 | 275-60 |
| Allapinin | 149 | 54385-00 | 154 | 56210-00 | 159 | 58035-00 |
| Total: | | 152570-90 | | 157345-40 | | 162233-80 |

$$I\,ij = \frac{n\,i}{N} \quad (3),$$

where: Iij – intensity factor of medicinal products of plant origin consumption;

n – the number of consumers who use the drug in this sample;

N – the total number of consumers in the sample (N=420 people) [6].

The results of the calculation of the intensity factors are given in Table 1.

Then based on the analysis of the patient's case histories, drug intake standards were determined (Xij fact).

Recommendations for the obtained values Xij fact as indicative of standards initially held their statistical processing on the coefficient of variation, which confirmed that the value of the average actual flow has little variation.

So the obtained values of Xij fact can be used as an approximate standard and used to determine the need for medicines in the treatment of cardiovascular diseases. The expected number of patients was determined by the method of average geometric parameters according to statistical data for the period 2011-2016:

$$T2 = \frac{Б3}{Б2} \quad (4)$$

$$Tcp = \sqrt[n]{T1 * T2 * T3 * T4 * T5} \quad (5),$$

where: Б1, Б2...Б5 – the number of patients with cardiovascular diseases for the first, second ... the 5th considered years in this hospital;

Tcp (middle) – average growth rate.

The expected (planned) number of patients is determined by the formula:

$$Bi = B3*Tmiddle \quad (6),$$

where: Bi – expected number of cardiac patients;

B3 – number of patients in the pre-planning period;

Tmid – average growth rate.

According to statistical data, the number of patients treated in the planned hospitals for the period 2006-2016 was:

- 2011year – 2630 people;
- 2012 year – 2560 people;
- 2013 year – 2631 people;
- 2014 year – 2664 people;
- 2015 year – 2690 people;
- 2016 year – 2720 people.

From 2011 to 2016, the number of patients treated was steadily increasing.

The increase in morbidity, which occurs, we associate with the increase in the proportion of older age groups in the total population of the country. We calculated the expected number of patients with this pathology for city hospitals [7].

Next the calculation of the need for each medicinal products of plant origin over the period 2015-2017 was carried out. The calculation took into account the period of treatment of 1 patient – 21 days. The results of the calculation are shown in Table 2.

## DISCUSSION

The educational goals of this study are fully achieved. The results of our work are original. The article has prospects for further study of cardiac drugs of plant origin. Health forecasting is a dynamic process and requires frequent updates. This can be done with novel techniques and data, taking into consideration the principles of health forecasting. The methodologies currently used involve time series analyses with smoothing or moving average models.

The horizons of health forecasting are important but not classified in the literature, and so the approaches used to forecasting various horizons have no common benchmarks to guide new health forecasts. Health forecasting is a valuable resource for enhancing and promoting health services provision; but it also has a number of drawbacks, which are related either to the data source, methodology or technology. This overview is presented to stimulate further discussions on standardizing health forecasting approaches and methods, so that it can be used as a tool to facilitate health care and health services delivery [1].

The activities which form an established part of the program of health departments and nonofficial health agencies are directed in relatively small degree to health supervision of persons of middle and old age.

This limitation is a natural consequence of the traditional restriction of public health services to those involved in the control of preventable disease. Thus, the changing age structure of the population may be expected to produce effects opposite in nature on public health and medical services.

Finally, an important approach to the solution of this broad problem is offered through the extension of research in the cause and control of the chronic diseases characteristic of advanced life. Effective control of certain of these diseases is in part dependent on the demonstration of the etiologic agents involved.

Opportunity should be provided for the appraisal of existing methods of diagnosis and treatment, and the exploration of new procedures designed to bring the chronic diseases under early control. But an equally fruitful field of research consists in the development of public health methods which will solve the unique problems involved in coordinating the control of the chronic diseases in the community health program [1, 2].

## CONCLUSIONS

1. Economic evaluation, together with studies of clinical effectiveness, allows a more rational allocation of available resources. Unfortunately, it should be noted that the low demand for pharmacoeconomic analysis results does not yet allow the full redistribution of the financial flow of budget funds to the full extent. In this regard, special attention should be paid to the profitability of the drug from the «cost-minimization» position, which should be evaluated in a comprehensive manner, taking into account the cost of inpatient and outpatient care.
2. The system of measures to improve the organization and improve the quality of therapeutic care for the population should include organizational and methodological issues of providing and managing the quality of medical care and drug provision.
3. The most important place in reforming the industry should be allocated to improving the outpatient and polyclinic service are increasing the efficiency of using material and human resources, introducing new progressive forms of work into practice.
4. The solution of these problems is determined by the wide introduction of fairly simple and massive measures to improve the living environment, the implementation of preventive measures to prevent the realization of the risk of developing cardiovascular diseases, giving the maximum results at minimum costs.
5. In the present conditions, it is important to standardize the structure, types and volumes of treatment and rehabilitation activities, technologies and results, and the functioning of the system of medical and social care institutions for patients.
6. Prevention of diseases should be based on the implementation of government programs for prevention and promotion of health. This will improve and actively use methods of early detection of social and economic factors, which is a threat of development of diseases of the cardiovascular system.

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ORIGINAL ARTICLE

# DYNAMICS OF GASTRIN LEVEL IN PATIENTS WITH DIABETES MELLITUS 2 TYPE AND CHRONIC GASTRITIS AFTER *HELICOBACTER PYLORI* ERADICATION THERAPY

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## ABSTRACT

**The aim:** To study the gastrin level dynamics in patients with diabetes mellitus (DM) 2 type and chronic gastritis (CG) on the background of antihelicobacter therapy (AHT).

**Materials and methods:** 60 patients with DM type 2 and HP-associated CG underwent examination. Patients were divided into two groups of 30 patients each: group 1 included patients who received only standard AHT, group 2 – patients who in addition to standard AHT received the drug SB (Normagut, company Mega) 2 capsules 2 times/day.

**Results:** According to our study results yeast SB, not only increase the HP eradication rate but together with the standard AHT contribute to the serum gastrin reduction, which is a gastric acid stimulator, which in turn leads to an improvement in the CG clinical course.

**Conclusions:** Patients with DM type 2 and CG associated with HP should include yeast SB to standard AHT as they reduce side effects from this treatment (by an average of 20%), increase the eradication frequency (by 10%), and also lead to significant decrease in serum gastrin (up to 82.15 ± 2.47 pg/ml).A decrease in serum gastrin levels in patients with HP-associated CG and DM type 2 leads to an improvement in the clinical course of the diseases, namely a decrease in nausea, diarrhea, abdominal pain, and discomfort incidence.

**KEY WORDS:** diabetes mellitus 2 type, chronic gastritis, *helicobacter pylori*, saccharomyces boulardii

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## INTRODUCTION

The gastrin plays the central role in the gastric acid secretion regulation, causing the histamine release from enterochromaffin-like cells (ECL), which in turn stimulates parietal cells to acid secretion. When the antral mucosa is infected by *Helicobacter pylori* (HP), it causes local alkalinization due to the ammonia (NH3) formation from urea under the HP urease action. The gastrin increased release can be caused by reduced acidity that is detected by receptors on D or G cells or both. The increased histamine release from ECL cells and increased acid secretion is caused by minor hypergastrinemia. Because gastrin is a very potent gastric acid secretion stimulator. [1]

The stomach was considered as an unsuitable environment for most microorganisms due to its acidic pH and peristaltic motility, but various commensal microorganisms can colonize the stomach forming a gastric niche. Recent data suggest that commensal gastric microbes or their metabolites affect the ability of HP to colonize the stomach and directly modulate its pathogenicity and carcinogenic potential. [2]

Probiotics are "live microorganisms that, when used in sufficient amount benefit the health of the host." Therefore, the microbial balance can be restored by probiotics, thus preventing the side effects associated with the use of antibiotics. In particular, this benefit may be useful in the treatment of HP when high antibiotics doses are required. [3,4]

The use of Saccharomyces boulardii (SB) increases the HP eradication frequency while reducing the side effects associated with the use of the antibiotic. Also, the use of yeast SB leads to normalization of intestinal wall permeability, microbiota restoration, improved immune function, decreased the pro-inflammatory immune response, and pro-inflammatory and anti-inflammatory cytokines balance. [5]

## THE AIM

To study the gastrin level dynamics in patients with diabetes mellitus (DM) 2 type and chronic gastritis (CG) on the background of antihelicobacter therapy (AHT).

## MATERIALS AND METHODS

60 patients with DM type 2 and HP-associated CG underwent examination in the endocrinology and gastroenterology department of Transcarpathia Regional Clinical Hospital named after A.Novak. The patient's mean age was 54.2 ± 2.3 years. The 32 (53.3%) women and 28 (46.7%) men were among the patients. All patients underwent general clinical trials according to local protocols.

Diagnosis of DM type 2 was done according to the International Diabetes Federation (IDF, 2005) recommendations, namely the determination of serum glucose on empty stomach and after 2 hours of carbohydrate load, which

**Table 1.** The gastrin level dynamics in patients with DM 2 type and HP-associated CG on the AHT background

| Gastrin level (pg/ml) | | |
|---|---|---|
| **Group 1 (n=30) – standard AHT** | | |
| Before AHT | 121,05±3,55 | |
| After 4 weeks | 102,12±2,02 (in patients with successful AHT) n=25 (83,3%) | 115,03±2,54 (in patients with unsuccessful AHT) n=5 (16,7%) |
| Group 2 (n=30) – standard AHT+SB (Normagut) | | |
| Before AHT | 119,23±2,62 | |
| After 4 weeks | 82,15±2,47* (in patients with successful AHT) n=28 (93,3%) | 110,01±4,42 (in patients with unsuccessful AHT) n=2 (6,7%) |
| Control group (n=20) – healthy patients | | |
| 50,95 | | |

*significant difference p<0,05

was performed using the glucose oxidant method. The DM severity was assessed by the glycosylated hemoglobin (HbA1c,%) level, which was determined by chromogenic analysis on a Sysmex 560 apparatus (Japan) using Siemens reagents.

HP was studied using a C13-urease respiratory test (C13-URT) (ZINTA, Hungary) as well as a rapid urease test (CLO-test) and fecal HP antigens determination (CITO TEST H. Pylori Ag, Pharmasco, Ukraine).

Serum gastrin levels were determined using an ELISA Gastrin-EIA test kit Cat. No CS001 30.

All patients were treated with standard (AHT) for 14 days: pantoprazole 40 mg + clarithromycin 500 mg + amoxicillin 1000 mg 2 times/day. Patients were divided into two groups of 30 patients each: group 1 included patients who received only standard AHT, group 2 – patients who in addition to standard AHT received the drug SB (Normagut, company Mega) 2 capsules 2 times/day.

The inclusion criterion in this study was the presence of a confirmed DM 2 type and HP-associated CG diagnosis.

Exclusion criteria in this study were patients with DM type 1 and AHT in the past or at the study time.

All studies were carried out with the patients` consent, and their methodology was consistent with the 1975 Declaration of Helsinki and its revision in 1983.

Scientific research is a fragment of state budget topic "Polymorphic pathology indiseases of the digestive system, peculiarities of pathogenesis, correction possibilities", state registration number: 0118U004365.

The results analysis and processing of the patient examination were performed using the computer program STATISTICA 10.0 (StatSoftInc, USA).

## RESULTS AND DISCUSSION

Gastrointestinal adverse reactions resulting from the use of AHT were more commonly found in group 1patients with diarrhea found in 40% of patients, bloating – 49%, nausea – 60%, and abdominal pain and discomfort – 33%; compared with group 2, where SB yeast was used with standard AHT, diarrhea was detected in 19% of patients, bloating– 21%, nausea – 33%, and abdominal pain and discomfort – 10%.

All patients were evaluated for serum gastrin levels before and 4 weeks after the AHT. The data obtained from the survey are shown in table 1 as the indicators arithmetic mean.

Analyzing the data from table 1, it was found that when carrying out AGT in both groups there was a decrease in serum gastrin levels after 4 weeks compared with its level before treatment in both groups, provided that AHT was successful. Thus, in 5 (16.7%) patients from group 1 AHT were unsuccessful compared with group 2, where AHT was unsuccessful only in 2 (6.7%) patients, respectively in these patients no change in gastrin level was found. A significant difference was found between gastrin levels in group 2, where patients were additionally receiving yeast SB with standard AHT, so gastrin levels were 119.23 ± 2.62 pg/ml before treatment and 82.15 ± 2.47 pg/ml ($p < 0.05$) subject to successful AHT. As for group 1, patients also showed a positive trend in gastrin levels in 4 weeks after the AHT, but these indicators are not statistically significant (before treatment – 121.05 ± 3.55 pg/ml and in 4 weeks – 102.12 ± 2.02 pg/ml).

According to studies, probiotics monotherapy can contribute to 14% of HP eradication, although from a clinical point of view this is an unsatisfactory indicator. However, this percentage is significantly higher than the placebo, meaning that a direct probiotics antibacterial effect against HP exists. [6]

After studying the yeast SB action, other researchers determined the level of successful HP eradication – 11.8%, thus, this indicates the reliable efficacy of this drug in HP-associated CG. [7]

According to our study results yeast SB, not only increase the HP eradication rate but together with the standard AHT contribute to the serum gastrin reduction, which is a gastric acid stimulator, which in turn leads to an improvement in the CG clinical course.

## CONCLUSIONS

1. Patients with DM type 2 and CG associated with HP should include yeast SB to standard AHT as they reduce side effects from this treatment (by an average of 20%), increase the eradication frequency (by 10%), and also lead to significant decrease in serum gastrin (up to 82.15 ± 2.47 pg/ml).
2. A decrease in serum gastrin levels in patients with HP-associated CG and DM type 2 leads to an improvement in the clinical course of the diseases, namely a decrease in nausea, diarrhea, abdominal pain, and discomfort incidence.

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ORIGINAL ARTICLE

# PSYCHOCULTURAL TRAINING OF SENIOR PUPILS TO ADEQUELY PERCEIVE THE MEDIA SPACE CHALLENGES

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## ABSTRACT

**The aim:** The research consists in the theoretical substantiation and experimental verification of the cultural and mental transformations of senior pupils under the influence of works of art, the media; highlighting the steps of forming the media literacy of pupils in the lessons of Ukrainian literature and in the after lessons in the process of independent activity of pupils to acquire their values and moral orientations.

**Materials and methods:** The complex of methods is used in the work: general scientific (analysis, synthesis, simile, comparison, systematization, generalization) and empirical (observations, conversations, questionnaires).

**Results**: It is established, that media tools have manipulative character in the minds of teens, which is both destructive and constructive and also carry propaganda of deceptive spirituality ; education and science are the main tools to counteract speculative juggling of information. The necessity of formation of media literacy, media competence, their theoretical and methodological foundations for the protection of senior students from emotional manipulation is substantiated. The author's vision of the definitions of "manipulation" of consciousness, "media education" is presented on the basis of research of scientific sources. Significant is the proposed steps to overcome stress as a result of emotional and cognitive transformations.

**Conclusions:** Educational activities of teachers, aimed at understanding of the media manipulation means by the pupils, the essential features of the art of words, will promote to preserve the health of pupils, serve the formation of their moral values, the formation of media literacy and media competence.

**KEY WORDS:** emotional manipulation; mental health; media tools; media literacy; Internet resourcesv

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## INTRODUCTION

Nowadays we live in an age of constant stress, so it is not accidentally emotional health and social behavior of pupils are getting important. Exactly on how much a person is able to protect himself in the conditions of information war and manipulation depends on his physical, moral state, civic position. Different ways of influencing a person, especially young people, are also reflected in a lack of understanding of the affectivity of the processes that provoke the distortion of messages, the "cult of impersonality". We also see the psychological features of senior pupils , in particular the reduction of stability of attention, the complexity of processing large amounts of information, dependence on gadgets, sensory and emotional hunger. Spiritual degradation, inhibition of mental development, inability to adjust in society become also negative factors. It is not about conformism but a healthy adaptation to society without losing your priorities, values and attitudes. It is not accidentally that health-saving technologies, the ability of everyone to recognize manipulation techniques to avoid the psychotraumatic impact, and to develop immunity for students from the stamped literature are acquiring importance.

The relevance of the problem is indicated by the fact that mental depression due to stress is compared with physical activity, it goes beyond the situational mood of the individual. As a result, we observe psychotherapy-oriented relationships among young people and burnout among adults, which occurs gradually, passing (according to M. Sitsynska) three stages: anxiety, resistance (resistance) and exhaustion [1]. Fatigue, failure, dissatisfaction, excessive responsibility, situations, identification of students with characters of electronic games, negative heroes of works become psycho-traumatic factors. They weaken and subdue the body. It is easy to manage and use young people who are not accustomed to combating information manipulation, negative circumstances. The media repeatedly emphasize the change in the roles of training (consultant – client), which causes misunderstanding of some and indignation of others, we also see in various talk shows and propaganda of deceptive spirituality, the illusion of omnipotence.

In our opinion, modern teenagers mostly read short texts from the Internet and do not want to get acquainted with program works in literature, the paper book competes with the electronic one. One way to solve the problem of reading

crisis and to return students to the art of words is a new model of social interaction, understanding the impact on students' tastes and preferences [2, p. 102]. All this reflects social phenomena and causes a need in the formation of media literacy of the recipients, the acquisition of experience of parity relations between the subjects of education.

The works of scientists point to the need to: a) take into account the psychological prerequisites for the use of media in order to form literary competence ; b) the organization of a new type of training, which would shift from trusting perception of getting information and accumulation of knowledge to a conscious analysis of the life and creative path of artists, their works.

For our study, understanding of the concepts of "media literacy", "media education" becomes important, because it is they that can protect students from biased perception and understanding of information, manipulation of their consciousness, mental stress and breakdowns. According to O. Fedorov, media education (media education) is the process of personality development with the help and on the material of mass media (media) with the purpose of forming a culture of communication from media, creative, communicative abilities, critical thinking, skills of full perception, interpretation, analysis and evaluation of media texts, teaching different forms of self-expression with the help of media technique [3, p. 27]. This makes it possible to develop students' creative abilities, gain their reading experience, make meaningful use of information sources and resources. If the student realizes for whom it is profitable to present this or that information in a certain light, the transformation of what he has heard or read or seen takes place.

Media literacy focuses on confident, and at the same time critical, use of information and communication technologies (ICTs) to create, search, process, share information at home, at school, in the public media, and in private communication. In the collective monograph under the scientific editorship of V. Rizun, media literacy refers to the level of media culture, which refers to the ability to use information and communication techniques, to express themselves and communicate with the help of media, to consciously perceive and critically interpret information, to separate reality from its virtual reality, that means to understand it designed by media sources to comprehend the power relations, myths, and types of control which they cultivate [4, p. 19]. Media education and media literacy predict that the pupil will learn continuously during the life, determine a promising goal and achieve it, choose the means to achieve it, build his own learning trajectory, acquire competencies, evaluate his own learning results, do reflection, control emotions while working with media sources and consciously approach to their evaluation.

The media competes for the audience, so they do not always use the democratic means and the truth of the presented information. Such competition requires knowledge of survival and creation in the information world, experimentation, active learning and problem solving. According to V. Rizun, media education should provide knowledge on how to: 1) analyze, critically think and create media texts; ) identify the sources of media texts, their political, social, commercial, cultural interests and context; 3) interpret media texts and values that carries the media itself; 4) to select the appropriate media to create and distribute their own media texts and to attract the interested audience; 5) enable free access to media for consumption and production of own media products [4, p. 9]. Selection of alternative sources of information, use of positive thinking, understanding of what reality is presented, what is concealed, how it is influenced, why it is given preference and for what reasons in the literature lessons and beyond, it enables the formation of media competence, polycompetence that motivates to meaningful learning.

Recipients are citizens of the world, they combine and represent different messages, decoding media texts, increase their field of knowledge, and generate models of behavior. Media Education is not training in the traditional sense. It predicts the presence of a dialectical approach, a sense of reading, empathy, experience. Key concepts of media education, as L. Masterman proves, include: definition, association, genre, selection, non-verbal communication, media language, naturalness and realism, audience, design, media perception, representation, rethinking, coding or decoding , emphasis, story, plot structure ideology, rhetoric, discourse (language, speech) and subjectivity [5, p. 41]. They enable us, as a joint project of teacher and student, to obtain multicultural education, to recognize cultural and anticultural phenomena in society, to realize our own creative potential, to build social relationships, which predict correspondance of the today challenges.

Now students are able to observe the explanation and interpretation of the same texts from different positions. In order to be media-competent, according to D. Kelneri, D. Shar, one must understand the subjectivity and prejudice of those who create the messages, as well as the content of social contexts. Along with this coding of subjectivity comes numerous readings of the text, decryption by different audiences in different contexts. Thus, the media are not neutral disseminators of information, because of the nature of construction and interpretation processes entail prejudjment and social influence [6, p. 12]. The possibility of the subjects of education to discuss and interpret the received information transforms them into active consumers who critically think, support or refute cultural discourses, discuss different films, programs for the purpose of education and getting experience, culture, cognitive component, immersed in the educational process, promoting certain ideas.

Based on the above mentioned positions, we understand the media education of students as part of the educational process, which predicts the use of productive teaching methods in order to understand the translation of political, cultural, social, ideological, economic media texts and awareness of their consequences and influences on psychics, manipulation, conducting dialogue at the personal and interpersonal levels, critical perception of information, aesthetic and spiritual education, acquisition of funda-

mental values, formation of outlook, mastering the means of self-expression with the help of media technicians and media means (traditional and new), as well as continuity in the acquisition of competencies in person and remotely, the integration of media in modern education, self-education.

Recipients of grades 10-11 in literature lessons revise expand their own system of life values, rethink contradictions of personal development, try to control their feelings. Emotions of man, aesthetic education, as notes A. Maslow, forms a special approach to learning, cognition, because higher excitement almost never go without trace [7, p. 189]. Sensual perception creates internal preconditions for the aesthetic development of the individual, which predicts a general erudition, acquaintance with literature in the national and world context through the use of ICT, media resources, but best when it is combined with comprehension, using the mental efforts of readers, because intellectual work enables students to acquire informational, literary and media competences, to understand the author's intention.

Exploring emotions, D. Desteno, R. Petti, D. Wegener argue that positive and negative moods increase the likelihood of future events. Their research demonstrates that not only positive emotions can affect a person. Sadness and anger are two different, negative emotions that differentially partially evaluate the likelihood of sad and angry future reactions, and also are an addition to supporting emotions as information [8, p. 405]. The future actions of the person depends on the mental state of the person, what information she hears and perceives. There are several options teenagers' reaction on negative one of them is denoted by resistance, the desire to find the truth, the other – by vulnerability, excessive exitements because of low likelihood of satisfaction, and there is one, that is accompanied by a desire to imitate. Subsequently, constant stress can directly affect a person's health, affect his or her well-being (emotions, apathy, fatigue, pressure increase, palpitations, anxiety, headache, irritation, anger, etc.) as well as indirectly (influence on actions). Sometimes we see that a child whose psyche is overwhelmed by negative emotions through computer games, anger, fears and doubts has a desire to act aggressively. As the information flow of the media repeatedly demonstrates such phenomena, children perceive them as normal. Only explanatory work and formation of media literacy skills can protect a child from emotional strain.

Modern teenagers have clip thinking, visualization is important for them. If pupils before demonstration of media text are provided also emotional commentary it will have a greater impact on the perception of auditory and visual images. Sensitivity to information, empathy will become much brighter. Emotional images from M. Namar's position cause strengthened parietal positives, starting from approximately 300 ms after the presentation stimulus. The significance of these reactions, however, depends on both internal (stimulated) and external (context-driven) factors [9]. The consonant is the thought of P. Valdesolo, who emphasizes that manipulations of emotional context form moral judgments [10]. The individual nature of the perception of media text influences the formation of personal spiritual and value orientations and aesthetic needs of students, so it is important to pay attention not only to the content, but also to the purpose of its presentation and the means which are used. Adjustment of pupils' emotions, promotion of the ability to filter information becomes a primary task of the teacher, because thanks to the art of words, he has levers of influence on the child and his mental state.

The necessity of using a differentiated emotional approach, the need to take into account the potential synergistic and oppositional effects of emotions that can occur in parallel, and the need to study their influence on regulation are emphasized by D. Desteno, J. Gross, and L. Kubzansky [11, p. 475]. The pupil has to cope both internally and externally, to perceive the world and to give it an evaluation. Ignoring of stressful situations will affect the unmanageable behavior of the student and the possibility of provoking psychological breakage. It is important for high school students to have their own ideas, beliefs, settings, values, attitudes, and use psychological protection mechanisms to reduce pressure and tension.

A person who is confident in his principled position, more difficult to get on the fake hook. However, the main cause of occurrence of the manipulation phenomenon is, according to Perls, in the eternal inner conflict of man between his desire for independence and independence, on the one hand, and the desire to find support in his environment – on the other [12, p. 332]. Teenagers are characterized by self-centeredness, unwillingness to listen to adults' thought, large-scale plans, aspirations for the risky and unknown, and friendly support, which is why they so strive to engage the adult world, demonstrate behavioral ease, peremptory judgements heard from the media, increased confidence. To some extent they do not pay attention to the fact that the information should be filtered.

Studying literature, reviewing media sources requires a meaningful, active, managed perception of artistic text or media text, creative analytical and synthetic activity, elaboration of it in order to form pupils' spiritual and value orientations and aesthetic needs. Whatever the level of sensory intelligence, manipulative technologies affect everyone, but with different power. Investigating the effect of emotional manipulation on men and women, R. Greve, L. Panebianko indicate that the mechanisms behind them differ depending by sex [13, p. 82]. For scientists, it became obvious that all people with higher levels of intelligence are capable of predicting emotional manipulation in the processing of social information, indirect aggression, but men also notice cognitive distortions. Women were revealed more sensitive to manipulation, able to detect primary psychopathic features and lower levels of social consciousness. However, for them emotional intelligence acted as a suppressor. For this reason, we understand manipulation as an informational, intellectual, psychological influence of a hidden nature, in which only one party of communication receives benefits and the other is encouraged to perform pre-planned actions by someone, inclined to accept others' beliefs intentionally or unintentionally.

Considering the commercial nature of contemporary writing, we observe double standards and coding, in which writers, journalists, critics, and publishers take part. The Ukrainian mass literature, from the point of view of S. Filonenko, is in a dual status: on the one hand, it is branded as waste paper, an opium for the people. On the other hand, it is thought as a desirable "guest from the future" [14, p. 378]. A single transmedia conglomerate was formed, where a literary work complements a television show, a blockbuster, a glossy magazine, a computer game, and does not claim a dominant position in it [14, p. 380]. Relatively speaking, mass literature is a universal category as the oldest representative of the media. The aesthetic value of such works is unrelated to the spread and the level of sales, since there is no demarcation board between the ugly and the beautiful, the culture and the anti-culture.

## THE AIM

Consists in disclosure the cultural and mental transformations of senior pupils under the influence of works of art, the media; elucidation of the steps of formation of media literacy of pupils at literature lessons and after lessons in the process of independent activity of pupils to acquire their values and moral orientations, equipping by the system of reader's actions in the expedient use of media.

Tasks – to give an idea of theoretical achievements of scientists in relation to media education, perception, emotions and stresses caused by the use of media, literature; to show positive and negative possibilities of use of works of art, media texts for perception, development of outlook of pupils, their mental health; display the results of the experiment.

## MATERIALS AND METHODS

For actualization the didactic aspects of the problem, we used methods of analysis of normative and scientific sources, systematic analysis and generalizations, the results of our own empirical researches, questionnaire materials, factor and criterion modeling of spheres of mental development of the child, created on the basis of qualimetric evaluation.

In order to check the levels of formation of media competence on the basis of the results of research and experiential learning it was develop that literature tasks that took into account the level of acquired reading skills, individual characteristics, the ability to perceive a representative system of pupils, their ability to see manipulative influences in works of art, doubtfulness of information, emotional coloring of the content, its sensationalism and secrecy of topic. Each task required the ability to think critically, use the means of ICT, the Internet, to argue own position, which contributed to a deeper understanding of the work of art, the author's position. For conducting of the experiment, it was suggested to study and elaborate art material from both the school curriculum and examples of mass literature, cinematography, mass media. The questionnaire, explanatory work, instructions and lessons on media competence of the pupils were conducted, matrices of evaluation of publications and videos were offered, training on the Internet resources was carried out.

## RESULTS AND DISCUSSION

For formation of media competence of students we offer the following steps: 1) arming the system of reading actions in the expedient use of media (analysis and synthesis of the content which had read and seen the formation of an independent view in the interpretation of media texts, works of art as a part of the media, the ability to see the influence of manipulation and personal-motivational effects, development of skills of independent critical thinking, interpretation of read and seen on the basis of context, posing questions to the author and to himself, meaningful "compression" of text, creative thinking activity, figurative-emotional perception, correlation of knowledge and reading skills), 2) superiority of intelligence over emotions, 3) acquisition of cultural values, own ideas, beliefs, settings, values, attitudes, 4) critical use of informational and communicative technologies, selecting the media to create and disseminate your own media texts 5) continuous learning, educational activity of teachers, 6) using psychological protection mechanisms to reduce pressure and tension, 8) use of a differentiated emotional approach. This can, over time, be a challenge to society, the media, and, on the other, to consolidate the efforts of learning subjects in acquisition of media literacy, and to improve education not only from the top-down, but also in the upward direction.

In order to make adequate decisions, to evaluate what has been heard, seen and read, it is necessary to have reader and life experience, intelligence must outweigh emotions. Their acquisition is promoted by lessons of literature, a detailed analysis of what has been read, although some writers do not prevent the child's stress, but rather reinforce it by using sensual juggling. This is evidenced by the literary discourse (T. Gundorova, V. Yerofieiev, M. Kundera, R. Musil) and the total format of interaction between mass media and mass literature, therefore for a group of pupils of the 11th experimental class with the basic level of literary and media competence, the task of finding features and methods of manipulation in classical composition was proposed. The results were given as a joint presentation on Google drive. The high school students found out that in the works of the school program there are psychological stresses and emotional manipulations: 1. Murder, drunkenness, robberies (the novel «Do Oxen Low When Mangers Are Full?» by P. Mirnyi and I. Bilyk), 2. Betrayal, manipulation of the male consciousness (the novel "Stolen Happiness" by I. Franko); 3. Family problems (the story "The Kaidasheva's Family" by I. Nechui-Levytskyi), 4. Sisterhood (the story "The Tale of the viburnum panpipe" by O. Zabuzhko), 5. Belief in "werewolves", "sorcerers" and other "unclean power" (story "Night on the eve of Ivan Kupala" by M. Gogol), 6. Social kitsch (poem "Aeneid" by I. Nechui-Levitskyi), 7. Stress-induced disorders of the psyche and behavior (Story

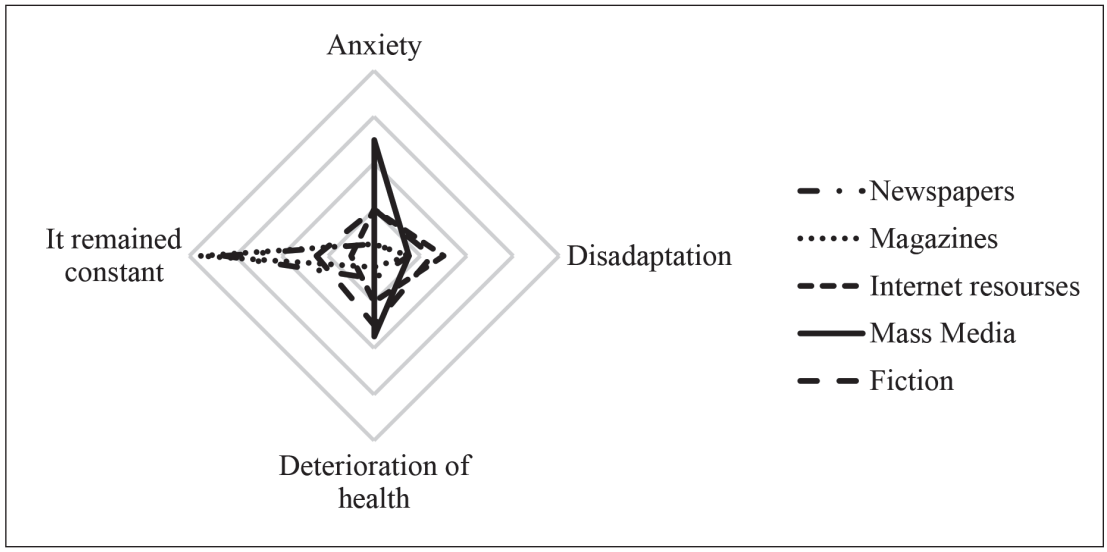


**Fig. 1.** The results of questionnaire in relation to the well-being of high school students while reading newspapers, magazines, works of art, viewing news in the form of a petal diagram.

of "Shadows of Forgotten Ancestors" by M. Kotsiubynskyi). Recipients noted that all of these works, through the management of readers' consciousness, the imposition of subjective thought, negative emotional coloring, affect the health of readers, and some fragments are to some extent unacceptable for study. In addition, not all senior pupils are able to critically evaluate a work, express an attitude about it, tell what they did not like, and admit the wrong decisions of heroes. The effect of a "wagon with a trailer" is created when the teacher persuades to read the composition because all have already done it. This usually leads to poor health of the students, holding them in doubts, anxious and humble position.

A group of schoolchildren with sufficient level of media competence needed to analyze foreign works of art in order to identify signs of hidden manipulative character and give examples in the form of mental cards, art scribing, and also to illuminate the influences on the emotional state during the reading of books. Pupils of grades 10-11 found out that the adventure novel of the occult writer Br. Stoker contains stories about the bloodthirsty vampire Count Dracula; novel "Goodbye, sadness!" F. Sagan – about fornication and fear of losing privileges; manipulations for the purpose of survival (K. Abe, novel "The Woman in the Sands", D. Steinbek, the novel "The Canning Row"); emotional burdens and tragic depreciation of life, desire for death to the native (F. Kafka, story "Reincarnation"), amnesia social, religious, cultural in the works of A. Merdok, U. Eko, G. Muller, O. Balzak, gothic entourage in novel A. Conan Doyle's The Hound of the Baskervilles; the myth in M. Bulgakov's novel "Master and Margarita", the absurdity and tragic stoicism in the novel "Plague" by A. Camiu; the hopelessness of the situation in F. Dostoievskyi's novel, "Crime and Punishment". Pupils testified that the constant keeping of the fans of these genres in the tension and fear in the future may affect the psyche and be accompanied by disorders, ethical decline, indifference to the problems of close people cultural transformations not in the better side. In the process of reading such works, readers felt lonely, betrayed, unhappy, unattractive, naive, too trusting. This was reflected in their emotional background. They gave their impressions in live broadcast, the results are shown in the table (Fig. 1).

According to the diagram, we are tracing that 50% of respondents in the experimental classes believe that media reports are most alarming Internet resources (games, news) and the aforementioned works of art – 20%; 5 and 5% reading youth newspapers and magazines; a feeling of inadaptability to life revealed 30% of respondents while working on sources of information which is in the Internet, 25% – while reading works of art, and the rest – 15%. Deterioration of state of health (physical, mental and working) contributes to the media (35%), reading literature with a depressive plot (30%), Internet resources (20%), newspapers (10%), magazines (5%). The least influence on emotional state or feelings of pleasure is felt by the students while reading magazines.

Pupils with high levels of media competence have been offered to find works in the contemporary literature that have different types of manipulation and to present in Table I, as well as in cinema – tapes that add excessive adrenaline and give examples of the asocial behavior that is demonstrated; transform their plots and present them in other genres by creating storytelling, as well as analyze well-known talk shows of Ukrainian television and identify means of manipulation by placing them in clusters (info sheets, pictochats, publications).

In the cinema, the recipients examined the pictures and revealed the following psychological effects: 1. Mad scientists in the films "Total recall" by L. Wiseman, "The Matrix" by L. Wachowski, E. Wachovski, 2. The spread of crime in the movie " Ocean's 8 " by G. Ross, 3. The killer woman ("Nikita" L. Besson), 4. The distorted understanding of love ("Doubtful love" by L. Weichzhy), 5. Shown destruction,

**Table I.** Mental and cultural transformations of readers under the influence of manipulations used in literature

| Work, author | Manipulation (content, method) | Impact on human health and / or morality and aesthetic consciousness |
|---|---|---|
| O. Buzina. «Ghoul Taras Shevchenko.» | The use of taken out of context facts and unproven data manipulation of writer's name. Anonymous authority. | Destruction of national pillars through misrepresentation. |
| Yu. Andrukhovych. Novel. "Perversion" | The identity of the person. Content socialization. | Formation of numerous psychic contradictions, complexes of inferiority and fears. |
| E. Pashkovskyi. Novel "Wolf Dawn". | Tragic collisions, aesthetization of dying. | Loneliness, homelessness, alienation of man. |
| I. Carpa. Novel."The Pearl Porn (The Supermarket of Solitude) " | Manipulation of the male consciousness, false glamor, zombie advertising. The spiral of silence. | A person feels himself a victim of progress, an escape from himself, depression. |
| A. Siniavskyi (Abram Terts). Novel. "Good Night" | Romanticization of camp life. The leader of thoughts. | Upturning of values and ideas, creation of prejudices. |
| A. Sierova. Novel, "The Rules of the Game' A. Krushelnytskyi. Story "Irena Olenska». | Fashion on feminism. Anonymous authority. | The imposition of defects |
| S. Zhadan Novel "Depesh Mod" | Recoding of cultural signs, social marasmus. Distraction of attantion. | An unhealthy desire of epatage |
| R. Ivanchuk. Novel "The Land of Irredent" | Political manipulation. Herd instinct. | Pessimism, despair. |
| L. Kostenko. The Notes of the Ukrainian Mad" | In a world of excessive misinformation, the hero is a hostage to the world's absurdities. The spread of information noise. | Hopelessness. Rethinking in Ukrainian coordinates. |
| I. Rozdobudko. The novel «Go over the darkness». The novel «Twelve, or the upbringing of a woman in unfit for life conditions.» | Manipulation of female consciousness; false glamor. Neurolinguistic programming. | Creates general dramatic mood. Creating the illusion of happiness. |
| S. Zhadan Novel ."Mesopotamia" | Love is monogamous / polygamous. Myth. | Creating the illusion of happiness. |
| T. Pinchon. "Lot 49 is exclaimed" | Ideological kitsch. Populism. Herd instinct | The pleasure of destruction. |
| A. Kokotiukha Novel. "The Dark Water" | Gothic poetics. Lame mind / tricky calculation. | Horror. Mental irritation. |
| L. .Denysenko Novel «24:33:42». | Philosophy / neurotic activity. Kitch. | Anxiety, stress. |
| S. Protsiuk Novel " Infection". | Infectiousness of the world. Fake. | The futility of pseudo-ideals. |

degeneration of the body ("American Mary" by Jen and Sylvia Soski), 6. A Reversal of Traditional Moral Values ("The Dark Knight Rises" by K. Nolan), 7. Massacres, speech of hate ("Psycho" by A. Hitchcock ) etc. Speculation, active manipulation of thoughts, the illusion that a person is able to make choices, games in giveaways and on emotions, muffling of alternative information, cacophony, which is run by the announcer-"conductor", propaganda, fakes, false heroes and pictures, the appearance of anonymous authorities, Fake headlines, jeans, agitation, advertising, deliberate suppression of information, pupils observed in various types of talk shows: entertaining, social, political ("Ukraine Says ", "Millionaire – hot seat", "Change wife", " Ukrainian top model "," Battle of the Extrasens "," Ukrainian Sensations ", etc.) as well as in the news. According to high school students, it prevents the independent perception of information, independent assessment of facts, allows psychological and mental stress, arouses imagination, causes the appearance of heart disease, as there are such ways of influence: suggestion, conformism (change of one's own opinion, on the position imposed by majority) example and imitation, persuasion (false arguments and facts), ideological influences (chauvinism, racism, separatism, sionism, primitive nationalism), socio-dynamics of culture (presentation of information fragments, borrowed from context).

To obtain the results of students' readiness to work with media texts, to determine the understanding of manipulation techniques by high school students, skills to defend themselves against their influence, a primary and repeated

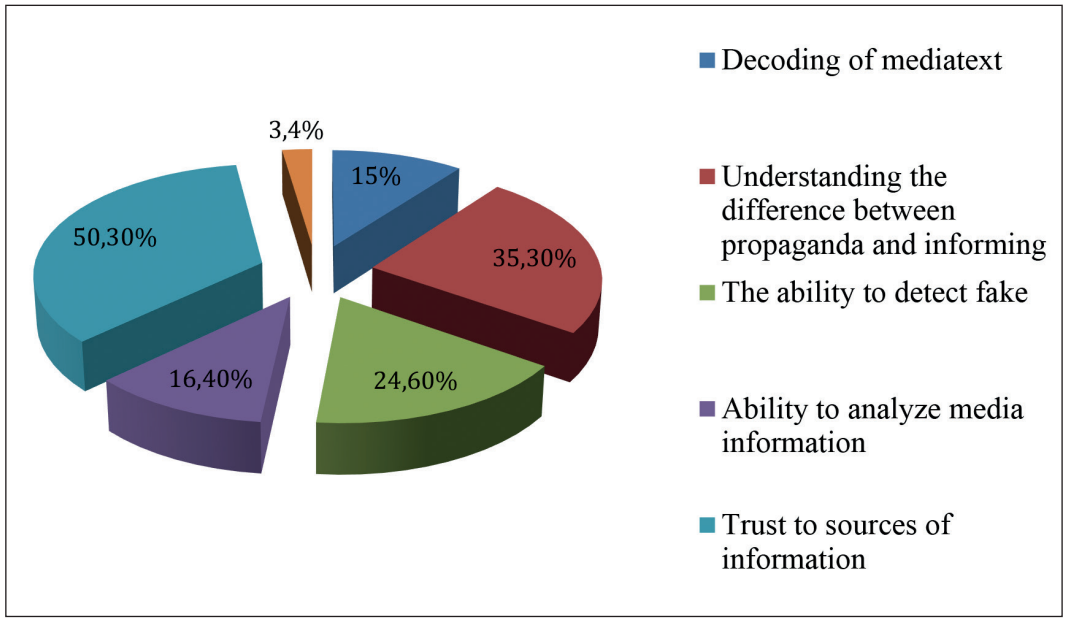


**Fig. 2.** Data of pupils' readiness to work with media texts (beginning of the experiment)

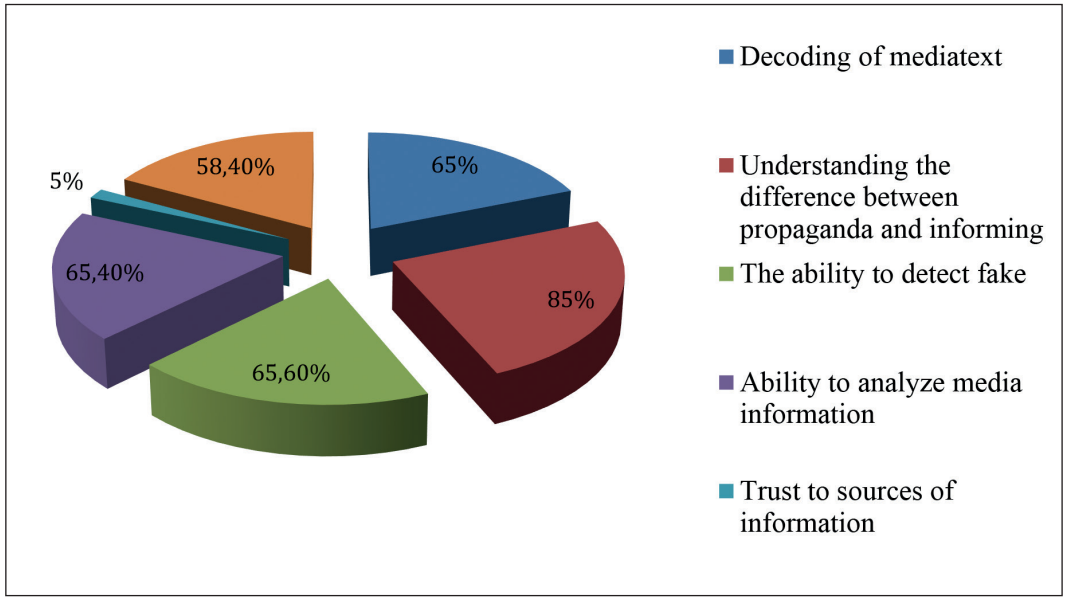


**Fig. 3.** Data of pupils' readiness to work with media texts (end of experiment)

survey was conducted in experimental classes. The following questions were offered in the questionnaires: 1. How to decode media text? 2. What sources of information are credible? 3. What is the difference between propaganda and informing? 4. How to analyze cinematic elements? 5. How to check a media expert? 6. Are you able to detect the fake independently? The survey results are presented in the diagrams.

To summarize, we highlight the availability of problem of understanding the advantages and disadvantages of using media in the process of teaching literature, the dependence of the perception of the world of high school pupils from its presentation by television and cinema, in magazines and newspapers, radio and the Internet, video games and mass literature. Having commercial support and political preference, media can juggle facts that affect people's minds. In this situation, children become hostages because they are not used to quickly analyze and interpret what they have seen and read, perceive everything with trust, and have not finally crystallized life values.

## CONCLUSIONS

Conducted experimental research of the mental and cultural transformations of readers as a result of manipulations in the media and literature revealed the following:

1. Theoretical works of scientists on media education, perception, emotions and stresses caused by the use of media, literature have been considered; positive and negative opportunities for the use of works of art, media texts for perception, development of pupils' outlook, and their mental health have been demonstrated on examples.
2. The author's vision of the definitions of "manipulation" by consciousness, "media education" have been presented on the basis of research of scientific sources.

3. Cultural, mental transformations of pupils of 10-11th grades occur under the influence of works of art, mass media. In order to effectively evaluate them, to avoid the impacts of manipulation, it is necessary to create media literacy.
4. The steps of formation of media literacy of pupils in literature lessons and after lessons in the process of independent activity of students for acquisition of their values and moral orientations, equipping the system of reader's actions in the expedient use of media have been outlined.
5. The results of the experiment have been shown, which indicate that the priority values of modern education should be: humanism, respect, human dignity, national identity, responsibility, etc.

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REVIEW ARTICLE

# THE THEORETICAL AND LEGAL BASIS FOR ENVIRONMENTAL RISK AS A POSSIBLE MEASUREMENT OF HARM TO THE ENVIRONMENT AND HUMAN HEALTH

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## ABSTRACT

**The aim:** To inquire into a theoretical and legal basis that regulates relevant areas and processes; use of certain objects, associated with ecological risks, which association, in turn, indicates the likelihood of conditions that can have adverse effects on the environment, human life and well-being.

**Materials and methods:** In this work we study statutory regulations and scientific positions of scholars regarding the above-mentioned issue. The study analyses generalized information from scientific journals employing scientific methods from a medical and legal perspective. This article is based on dialectical, comparative, analytic, synthetic, and comprehensive research methods.

**Conclusions:** The importance of the issue of environmental risks reflects the need in solving the problem of coexistence between human beings and nature. The analysis of theoretical and legal basis within the outlined framework will allow detecting the gaps and will help to understand in what way they are surmountable while regulating the stressors associated with ecological risks, on one hand, and, on another hand – the possible consequences, in order to prevent and eliminate them as promptly as possible and, thus, minimize their adverse effects on the environment and the health of the population.

**KEY WORDS:** risk, environmental risk, environmental risk assessment, health, natural environment, environment

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## INTRODUCTION

The world is facing multiple health challenges that range from outbreaks of vaccine-preventable diseases like measles and diphtheria, increasing reports of drug-resistant pathogens, growing rates of obesity and physical inactivity to the health impacts of environmental pollution and climate change and multiple humanitarian crises [1]. Since a healthy environment is a prerequisite for a healthy population, and environmental factors underlie the significant burden in terms of mortality and morbidity in the developing world. It requires a holistic, comprehensive and integrated approach to health and environment to protect both the environment and public health [2]. Accordingly, the study of ecological risks issue is crucial for developing the necessary approaches that can eliminate possible negative consequences. Some issues concerning the impact of certain stressors on the environment, in general, and therefore on the health of the population, in particular, have been covered by individual authors [3-8], but it is the outlined question itself that needs more in-depth study. Environmental problems and impacts continue to pose significant risks for human health and well-being, whereas measures to improve the state of the environment can be beneficial (Preamble, para. 25 Decision No 1386/2013/EU of the European Parliament and of the Council of 20 November 2013 on a General Union Environment Action Programme to 2020 'Living well, within the limits of our planet' [9]).

Human health and well-being are intimately linked to the state of the environment. Good quality natural environments can provide multiple benefits to physical, mental, and social well-being. However, environmental deterioration – such as that caused by air and water pollution, noise, radiation, chemicals or biological agents – can have adverse effects on health [10, p. 115]. Achieving a healthy and sustainable environment is a key ingredient for preventing diseases and enabling viable health care [11, p. 1]. In other words, the appropriate environmental conditions, as a result of certain circumstances, among other things, are related to in-deep comprehension of ecological risk, namely, its stressors and potential consequences, and thus, directly or indirectly, reflect on the environment and human health. Risk is defined as the probability that a substance or situation will produce harm under specified conditions. Risk is a combination of two factors: • the probability that an adverse event will occur (such as a specific disease or type of injury); • the consequences of the adverse event. Risk encompasses impacts on public health and on the environment and arises from exposure and hazard. Risk does not exist if exposure to a harmful substance or situation does not or will not occur. Hazard is determined by whether a particular substance or situation

has the potential to cause harmful effects [12, p. 1].

The issue of environmental risk study is important for understanding the likelihood of negative effects on the environment in general, which, in turn, might, directly or indirectly, affect human health. There are currently many contaminated sites in the Community, posing significant health risks, and the loss of biodiversity has dramatically accelerated over the last decades. Failure to act could result in increased site contamination and greater loss of biodiversity in the future. Preventing and remedying, insofar as is possible, environmental damage contributes to implementing the objectives and principles of the Community's environment policy as set out in the Treaty (Preamble, para.1) Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage [13]). The impact of environmental risk factors on health are extremely varied and complex in both severity and clinical significance. For example, the effects of environmental degradation on human health can range from death caused by cancer due to air pollution to psychological problems resulting from noise [14]. In recent years ecological risks (threats to the health and productivity of species and ecosystems) have also arisen as a topic of great public concern, in parallel with heightened attention to resource sustainability and concern over environmental degradation [15, p. 575]. In particular, achieving the 2050 vision, it focuses on three key areas: protecting the natural capital that supports economic prosperity and human well-being; stimulating resource-efficient, low-carbon economic and social development; safeguarding people from environmental health risks [10, p. 10]. Therefore, the study of outlined issues is quite topical in the context of a comprehensive understanding of potentially negative consequences and correlative causing factors.

## THE AIM

To inquire into a theoretical and legal basis that regulates relevant areas and processes; use of certain objects, associated with ecological risks, which association, in turn, indicates the likelihood of conditions that can have adverse effects on the environment, human life and well-being.

## MATERIALS AND METHODS

For a comprehensive study of the outlined topic we need to define the notion of ecological risk. In particular, as stated in para. 15 part 1 art. 3 Directive 2012/18/EU of the European Parliament and of the Council of 4 July 2012 on the control of major-accident hazards involving dangerous substances, amending and subsequently repealing Council Directive 96/82/E [16]: 'risk' means the likelihood of a specific effect occurring within a specified period or in specified circumstances. Risk – the probability of a specific outcome, generally adverse, given a particular set of conditions [12, p. 62]. In turn, according to para. 4 'Environmental risk' Division 2 'Definitions' Chapter 1 'General Provisions' General Part of the Environmental Code Act Passed: 'environmental risk' means the possibility of occurrence of an environmental nuisance that needs to be reduced [17]. In addition, as noted in dictionary sources, environmental risk – actual or potential threat of adverse effects on living organisms and environment by effluents, emissions, wastes, resource depletion, etc., arising out of an organization's activities [18]. Environmental risk – the risk associated with economic or administrative consequences of slow or catastrophic environmental pollution [19]. The environmental risks to health are defined as all the external physical, chemical, biological, and work-related factors that affect a person's health, excluding factors in natural environments that cannot reasonably be modified [20].

Thus, having regard to the above, we can sum up that ecological risk is associated with adverse effects on the environment in general and nature in particular, which can also affect human health negatively. Environmental risk factors, such as air, water and soil pollution, chemical exposures, climate change and ultraviolet radiation, contribute to more than 100 diseases and injuries [21]. Environmental hazards increase the risk of cancer, heart disease, asthma, and many other illnesses. These hazards can be physical, such as pollution, toxic chemicals, and food contaminants, or they can be social, such as dangerous work, poor housing conditions, urban sprawl, and poverty [22]. Accordingly, the source of the stressors can be different materials, substances or circumstances, which, due to its nature, are able to become factors causing harm.

## REVIEW AND DISCUSSION

While studying the nature of ecological risk, let's pay attention to its components. This will help us to understand it's essence better.

Firstly, the ecological risk is associated with certain factors, which indicate its existence. At every stage of their life – from conception to death – organisms are exposed to a multitude of environmental factors, some of which are associated with severe health risks [23]. These are the factors that have a natural or man-made origin and create a certain hazard. Environmental risk and protective factors encompass a broad range of factors related to socio-economic, ethnocultural, political, and policy conditions, as well as factors related to the natural and built environment [24]. Some are created by man through the introduction of new technology, product, or chemical, while others, such as natural hazards, result from natural processes that happen to interact with human activities and settlements. Some can be reasonably well anticipated, such as flooding in a valley or pollution from an industrial smelter. Others are wholly unsuspected effects at the time the technology or activity was developed, such as the possible effects on the earth's ozone layer of fluorocarbon sprays or nitrogen fertilizers [25, p. 3]. According to some authors, the actualization of ecological risks occurs as a result of exposure to harmful factors, they cause accidents, which interfere with normal living conditions [26, p. 157]. Environmental risk factors

cover a wide range of topics such as social, economic, cultural and political factors as well as physical, chemical and biological factors. Examples include access to clean water and sanitation; risks in the workplace; air pollution; social settings [27]. Environmental risks to health include pollution, radiation, noise, land use patterns, work environment, and climate change [20].

Among environmental risks, there are such factors causing diseases: pollution; microbes in the air, water, or soil; contaminants in food; weather conditions (e.g. droughts, heat waves); natural disasters (e.g. hurricanes, earthquakes, floods); pesticides and other chemicals; pests and parasites; radiation; poverty; lack of access to health care [22]. For instance, as stated in Preamble, para.6 Directive 2012/18/EU of the European Parliament and of the Council of 4 July 2012 on the control of major-accident hazards involving dangerous substances, amending and subsequently repealing Council Directive 96/82/EC [16]: major accidents can have consequences beyond frontiers, and the ecological and economic costs of an accident are borne not only by the establishment affected but also by the Member States concerned. It is therefore necessary to establish and apply safety and risk-reduction measures to prevent possible accidents, to reduce the risk of accidents occurring, and to minimize the effects if they do occur, thereby making it possible to ensure a high level of protection throughout the Union. In Preamble, para. 3 Directive 2007/60/EC of the European Parliament and of the Council of 23 October 2007 on the assessment and management of flood risks [28]: it is feasible and desirable to reduce the risk of adverse consequences, especially for human health and life, the environment, cultural heritage, economic activity and infrastructure associated with floods. However, measures to reduce these risks should, as far as possible, be coordinated throughout a river basin if they are to be effective. Therefore, the occurrence of ecological risks is connected with different areas, processes and objects, that can harm the life and the health of the population as well as the environment in general.

Secondly, there are might be relevant consequences which will have a negative impact on the environment, life and health of the population. Consequence (or impact) – the outcome (of an event), expressed qualitatively in terms of the level of impact. Consequences can be measured in terms of economic, social, environmental or other impacts [29]. Environmental health hazards affecting human health are most commonly classified as chemical, biological, physical, mechanical, and psychological [30, p. 14]. Their occurrence depends on the very factors that are their precursors. Accordingly, those two components are closely interrelated, namely, the clearer the origin of the potential hazard, the more possible it will be to assess the likely consequences. Environmental health is an area of growing concern due to major global environmental changes and an increase in established links between several diseases and environmental exposures [31]. Hence, for the study of the concept of ecological risk this assessment is quite important. For instance, in Preamble, para. 9. Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified microorganisms [32] is stated that the precise nature and scale of risks associated with the contained use of GMMs are not yet fully known and the risk involved must be assessed on a case-by-case basis. In order to evaluate the risk to human health and the environment, it is necessary to lay down requirements for risk assessment. In Preamble, para. 22 Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC [33]: where a Member State, the Commission or the marketing authorization holder considers that there are reasons to believe that a veterinary medicinal product could present a potential serious risk to human or animal health or to the environment, a scientific evaluation of the product should be undertaken at Union level, leading to a single decision on the area of disagreement, binding on the relevant Member States, and taken on the basis of an overall benefit-risk assessment. For all new applications for marketing authorization, environmental risk assessments should be mandatory and should consist of two phases. In the first phase, the extent of environmental exposure to the product, its active substances and other constituents should be estimated, while in the second phase the effects of the active residue should be assessed (Preamble, para. 31).

Environmental risk assessment is a process for estimating the likelihood or probability of an adverse outcome or event due to pressures or changes in environmental conditions resulting from human activities [34, p. iii]. Environmental risk assessment is the process undertaken to identify, evaluate and apply mitigation and control measures to the potential environmental risk of proposed development [35, p. 6-1].

According to some authors, assessment of the risk results in developing certain understanding regarding the classification of stressors (causing factors) and possible consequences of their impact [36, p. 52]. Classification criteria can have different origin, for example, by a certain area of impact, the time interval of occurrence, existence and eliminating of consequences, the priority of objects of relevant impact and indirect impact on other objects, etc. The process of risk assessment is based on those classification criteria, which, on one hand, allows us to have an in-depth approach to such procedure, and on the other hand, to get more precise forecasts and apply the results in other cases too, as fully as it is possible.

Risk assessment is defining as human exposure to environmental hazards causing a potential threat to adverse health effects [37]. Risk assessment is where the severity of the hazard and its potential outcomes are considered in conjunction with other factors including the level of exposure and the number of persons exposed and the risk of that hazard being realized [38]. Risk assessment – an organized process used to describe and estimate the likelihood of adverse health outcomes from environmental exposures to chemicals. The four steps are hazard identification, dose-response assessment, exposure assessment,

and risk characterization [12, p. 61]. Environmental risk assessment means the forecast for the likelihood that particular adverse consequences, considering the nature of their origin, will occur for the environment in general as well as for human health in particular, which should contribute to the development of appropriate measures to prevent their occurrence and occurrence of the disease, and the choice of appropriate treatment in the case.

Referring to judicial practice in the outlined framework, the improper evaluation of the ecological risks itself or neglecting it at all leads to the violation of human rights and adverse effects on the environment and, accordingly, on human health.

For instance, case of Guerra and Others v. Italy. This case dealt with the failure to provide the local population with information about risk factors and how to proceed in the event of an accident at a nearby chemical factory. The question was whether national authorities had taken the necessary steps to ensure effective protection of the applicants' right to respect for their private and family life. Severe environmental pollution could affect the individuals' well-being and prevent them from enjoying their homes in such a way as to affect their private and family life adversely. The applicants had waited, right up until the production of fertilizers had ceased in 1994, for essential information that would have enabled them to assess risks they and their families might run if they continued to live around the factory. The court awarded each applicant a specific sum as non-pecuniary damage [39]. In the case of Tetar v. Romania the Court observed that where pollution or noise interfered with a person's well-being, a claim could be brought under article 8 (right to respect for private and family life)and that the existence of a serious and material risk for the applicants' health and well-being entailed a duty on the part of the State to assess the risks, both at the time it granted the operating permit and subsequent to the accident, and to take the appropriate measures. The European Court of Human Rights held that there had been a violation of the right to respect for private and family life on account of the Romanian authorities' failed to protect the applicants who lived in the vicinity of the Baia Mare Aurul gold mine [40].

## CONCLUSIONS

Thus, the study of the components in the concept of 'ecological risk' and their appropriate legal regulation is essential, on one hand, for determining specific factors related to the likelihood of the negative consequences, and on the other – understanding their nature we can minimize the negative impact in case of their occurrence by implementing approupriate preventative measures.

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REVIEW ARTICLE

# HEMATURIA AND OTHER KINDS OF BLEEDINGS ON NON-VITAMIN K ANTAGONIST ORAL ANTICOAGULANTS IN PATIENTS WITH ATRIAL FIBRILLATION: AN UPDATED OVERVIEW ON OCCURRENCE, PATHOMECHANISMS AND MANAGEMENT

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**ABSTRACT**

Non-vitamin K antagonist oral anticoagulants (NOACs) are currently recommended for oral anticoagulation in patients with non-valvular atrial fibrillation. In the setting, NOACs effectively prevent from stroke and systemic embolic events. In spite of the favorable safety profile of NOACs when compared with vitamin K antagonists, the use of any kind of anticoagulation is associated with an increased risk of bleeding. However, there is still a lack of direct comparisons of effectiveness and safety among NOACs. The results of indirect comparisons and meta-analyses suggest that the risk of various types of hemorrhagic complications differ among the particular NOACs. Management of bleeding in patients under NOAC therapy can be challenging because of limited availability of antidotes and the lack of routine laboratory test monitoring the NOAC anticoagulant effect. In case of life-threatening or critical site bleeding, reversal of NOAC anticoagulant activity is essential together with immediate implementation of causative treatment. Moreover, some patients on chronic NOAC therapy may require urgent surgery or invasive procedures. Specific reversal agents for NOACs have been developed, i.e. more widely available idarucizumab for the factor IIa inhibitor (dabigatran) and andexanet alfa for the factor Xa inhibitors (rivaroxaban, apixaban, edoxaban) with limited availability. This review summarizes the occurrence and management of NOAC-related bleeding complications with a particular emphasis on hematuria.

**KEY WORDS:** bleeding, non-vitamin K antagonist oral anticoagulants, idarucizumab, andexanet alfa

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## INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia [1]. AF is associated with increased risk of thromboembolic events, heart failure, death and hospitalization [2]. In AF patients, OAC (oral anticoagulant) therapy is highly effective for ischemic stroke prevention. However, warfarin and other vitamin K antagonists (VKAs) use can be limited by narrow therapeutic index, variability in dose-response among patients and great number of drug or food interaction.

Non-vitamin K antagonist oral anticoagulants (NOACs) directly inhibit thrombin (dabigatran) or activated factor X (rivaroxaban, apixaban, edoxaban). NOACs have predictable pharmacodynamics and pharmacokinetics and display rapid onset and offset of action. Their use in non-valvular AF therapy is still increasing. According to the guidelines of European Society of Cardiology, NOACs are recommended as the first choice anticoagulants in most of patients with non-valvular AF and one or more stroke risk factors [3]. Four phase III randomized clinical trials showed that NOACs are at least as safe and effective as VKAs [4-7].

The use of OACs, both VKAs and NOACs, is associated with bleeding risk, including major bleeding, intracranial hemorrhage (ICH), gastrointestinal (GI) and urinary bleeding. Generally, NOACs therapy have been shown to significantly reduce the rate of intracranial hemorrhage compared with warfarin. However, these drugs may increase the risk of gastrointestinal bleeding. NOACs differ in their degree of renal excretion, half-life times, bioavailability and metabolism. There are no head-to-head clinical trials comparing NOAC therapies. Evidence from indirect comparisons should be carefully assessed.

## THE AIM

The aim of this review was to describe the occurrence, pathomechanisms and management of hemorrhagic complications in patients with AF treated with NOACs. We decided to pay a particular attention to hematuria as this kind of bleeding complication is common and so far only few papers have addressed this topic.

## REVIEW AND DISCUSSION

### HEMATURIA

One of the most frequent site of bleeding during OAC therapy is genitourinary tract. Macroscopic hematuria occurs in 2-24% of these patients [8]. Previous studies have emphasized the clinical significance of hematuria in patients on OAC therapy. OACs usually do not induce *de novo* hematuria, but they may have a negative effect on the intensity and duration of hematuria from other etiologies. OAC-related bleeding is often diagnosed in individuals with underlying genitourinary pathology (3-82%), such as malignancy, prostate diseases, infection, urolithiasis or congenital anomaly [9]. Among AF patients with hematuria, warfarin use might be associated with a higher prevalence and early detection of genitourinary cancer [10]. In contrast, some authors suggest that warfarin might have a protective effect on some types of cancer development, especially prostate cancer, but not renal or bladder cancer [11].

Moreover, the effect of NOACs versus warfarin on genitourinary bleeding may differ. A meta-analysis of over 175,000 patients on OACs demonstrated that the overall probability of visible hematuria was 26.7%. Warfarin therapy was linked to an increased risk for visible hematuria, but major hematuria was more common in patients receiving NOAC. Among NOACs, dabigatran was the most likely to cause major hematuria compared to warfarin (37% vs. 0.2%). Urologic pathology was found in 44% patients with hematuria, malignancy in 14% [12]. These observations indicate the importance of complete urologic evaluation for hematuria, including upper tract imaging and cystoscopy.

### INTRACRANIAL HEMORRHAGE

Intracranial hemorrhage is one of the most devastating OAC therapy complications associated with high risk of mortality and severe disability. Previous studies confirmed that warfarin therapy is linked with more hematoma expansion and higher mortality than spontaneous ICH [13]. The substantial benefit of NOAC therapy is driven by effective protection against hemorrhagic stroke. A meta-analysis of phase III trials showed that NOACs reduce the risk of ICH by half [14]. Another meta-analysis indicated that higher doses of NOACs might be associated with increased likelihood of ICH [15]. According to the results of the phase III trials, each NOAC was associated with lower rates of intracranial hemorrhage compared with warfarin. However, in these studies there have been only limited data on potential association between prior NOAC use and ICH outcome. Among patients with ICH, prior NOAC or warfarin therapy was associated with higher in-hospital mortality compared with patients with no prior use of anticoagulants. Prior use of NOACs was associated with lower risk of in-hospital mortality and better in-hospital outcome compared with prior use of warfarin [16].

The RE-LY (*Randomized Evaluation of Long-Term Anticoagulation Therapy*) trial showed that dabigatran 150 mg twice daily and dabigatran 110 mg twice daily reduced the relative risk of ICH (relative risk [RR] 0.26; 95% confidence interval [CI] 0.14-0.49 and RR 0.31; 95% CI 0.17-0.56, respectively). The risk of ICH was lower with both dabigatran doses compared with warfarin group irrespective of age [17]. This observation indicates that the relationship between age and anticoagulant treatment is not only a pharmacokinetic interaction. This findings support hypothesis that the lack of NOAC interference of VIIa-Tissue Factor (TF) complex formation might be associated with lower incidence rates of ICH compared with warfarin. Warfarin and other VKAs inhibit the synthesis of vitamin K-dependent clotting factors, including factor II (prothrombin), VII, IX and X. Plasma clotting factor VII is the natural ligand of TF (the cellular transmembrane receptor for factor VIIa). Factor VIIa-TF complex formation triggers the coagulation cascade. High TF expression in vital organs, such as the lungs, brain, heart, testis, uterus, and placenta provides addition protection against hemorrhage.

Some data suggest potential role of cerebral microbleeds neuroimaging in identifying patients who might net harm from oral anticoagulation. In the CROMIS-2 trial, in 1447 patients with AF anticoagulated after ischemic stroke or transient ischemic attack, cerebral microbleeds were independent risk factor for symptomatic intracranial hemorrhage [18]. In a meta-analysis of cohort studies including individuals with recent ischemic stroke and documented AF, the presence of at least 5 cerebral microbleeds was associated with high ICH risk. However, it remains uncertain how this knowledge might impact clinical practice [19].

### MAJOR BLEEDING

The comparison of major bleeding events from the clinical trials between particular NOAC and VKA is limited by differences in the definition of major bleeding. The RE-LY trial enrolled 18,133 patients. The rate of major bleeding (defined as a reduction in the hemoglobin concentration of at least 20 g per liter, transfusion of at least 2 units of blood or symptomatic bleeding in a critical area or organ) among the patients in the warfarin group was 3.36% per year, compared with 2.71% in the group receiving 110 mg dabigatran (p=0.003) and 3.11% per year in the group receiving 150 mg dabigatran (p=0.31). Life-threatening bleeding occurred more frequently (1.8% per year) in patients in the warfarin group as in those receiving dabigatran 110 mg (1.22% per year, p≤0.001) and dabigatran 150mg (1.45% per year, p=0.04) [6]. In the ROCKET-AF (*Rivaroxaban Once-daily oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation*) trial, the rates of major bleeding (defined as clinically overt bleeding associated with any of following: fatal outcome, involvement of a critical anatomic site, fall in hemoglobin concentration ≥2 g/dL, transfusion of ≥2 units of whole blood or packed red blood cells, or permanent disability) in the warfarin and

**Table 1.** Comparison of pharmacological characteristics of NOACs.

| Target | Dabigatran | Rivaroxaban | Apiksaban | Edoxaban |
|---|---|---|---|---|
| Prodrug | Yes | No | No | No |
| Oral bioavailability (%) | 7 | 66 | 50 | 68 |
| Mean half-time (h) | 14-18 | 7-13 | 12 | 50 |
| Renal clearance (%) | 85 | 33 | 27 | 50 |
| Plasma protein binding (%) | 33 | 95 | 87 | 99 |
| Potential interactions | P-glycoprotein drug modulating drugs | CYP3A4, P-glycoprotein modulating drugs | CYP3A4, P-glycoprotein modulating drugs | CYP2C9, CYP2C8, 2C18, 2C19, 1A2, 3A4 modulating drugs |
| Metabolism | Conjugation | Oxidation and hydrolysis | Oxidation and conjugation | Hydrolysis, unchanged (70%) |
| Dosing in AF | 150 mg twice daily or 110 mg twice daily (patients ≥80 years old / >75 years old (ESC) or concomitant use of verapamil or amiodarone) | 20 mg once daily or 15 mg once daily (patients with CrCl 30-49 mL/min) | 5 mg twice daily or 2.5 mg twice daily (if at least two of following: age≥80 years, body weight≤60 kg or serum creatinine level ≥1.5 mg/dL (133 µg/L) | 60 mg once daily or 30 mg once daily (patients with CrCl 30-50 mL/min, body weight≤60 kg or concomitant use of verapamil or quinidine or dronedarone) |

Note: AF – atrial fibrillation; CrCl – creatinine clearance; CYP – cytochrome P450; ESC – European Society of Cardiology; NOACs – non-vitamin K antagonist oral anticoagulants

rivaroxaban groups were similar (5.6% vs 5.4%, p=0.58) with significantly lower rates of fatal bleeding in the rivaroxaban group (0.4% vs 0.8%, respectively; p=0.003) [5]. In the ARISTOTLE (*Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation*) trial, apixaban reduced the risk of major hemorrhage by 31% compared with warfarin (hazard ratio [HR] 0.69; 95% CI 0.60-0.80; p<0.001). Major hemorrhage occurred more common in older patients, those with history of prior myocardial infarction, prior hemorrhage, prior stroke or transient ischemic attack, systemic embolism, diabetes or hypertension. Renal function impairment and lower hematocrit level were also more frequent among patients who sustained a major bleeding [4].

In the ENGAGE AF-TIMI 48 (*Effective Anticoagulation with Factor Xa Next Generation in Atrial Fibrillation–Thrombolysis in Myocardial Infarction 48*) study, the rate of major bleeding (as defined by the International Society of Thrombosis and Haemostasis) was 3.4 % with warfarin versus 1.61% (p<0.001) with low-dose edoxaban and 2.75% (p<0.001) with high dose edoxaban [7]. The risk of major bleeding in clinical trials was significantly lower with dabigatran 110 mg twice daily, apixaban twice daily and edoxaban once daily compared with warfarin. Similar observations were made by other authors. In real-world data, Yao e*t al.* using a large US insurance database found that in patients with AF apixaban was associated with lower risks of both stroke and major bleeding, dabigatran was associated with similar of stroke and lower risk of major bleeding, and rivaroxaban was associated with similar risks of both stroke and major bleeding in comparison to warfarin [20]. Also in a cohort of 19,713 newly anticoagulated AF patients, a significantly lower rate of major bleeding were observed on dabigatran or apixaban therapy when compared with warfarin treatment [21]. This results are worth emphasizing because the risk of major bleeding is higher in the early phase of anticoagulation therapy.

## GASTROINTESTINAL BLEEDING

In general, NOAC therapy is associated with an increased risk of GI bleeding compared with warfarin, with apixaban being the exception. Several local and systematic mechanisms lead to gastrointestinal complications of NOAC treatment. That might be the consequence of differences in bioavailability between these drugs and warfarin and longer NOACs persistence in the gastrointestinal tract (Table 1). The bioavailability of warfarin is 97%, while the mean bioavailability of NOACs is substantial lower (dabigatran 7%, rivaroxaban 66%, apixaban 50% and edoxaban 68%) [22]. Moreover, the NOACs may also inhibit GI mucosal healing. Old age, concomitant use of antiplatelet drugs, hepatic or renal dysfunction are additional risk factors of NOAC-related GI bleeding.

Some data suggest that dosing of novel anticoagulants can affect the incidence of bleeding. Once daily administration of rivaroxaban offer higher peak level than apixaban 5 mg twice daily and may lead to higher risk of bleeding despite the fact that both these drugs inhibit factor Xa, are administrated in active form, and have comparable bioavailability. The annual incidence of GI bleeding in patients receiving NOACs is up to 2% with rivaroxaban which is associated with highest risk. In the ROCKET-AF trial, the rate of major bleeding from a gastrointestinal site was significantly higher among the patients receiving rivaroxaban once daily compared with the warfarin group (3.2%, n=224 *vs.* 2.2%, n=154; p<0.001) [5]. In the RE-LY trial, the rate of major GI

bleeding was 1.12% per year in the warfarin group, compared with 1.51% per year in the patients receiving 150 mg dabigatran (p<0.001) and 1.02% in patients receiving 110 mg dabigatran (p=0.43) [6]. In the ARISTOTLE trial, there was no significant difference between the rates of major GI bleeding in the apixaban group and the warfarin group (n=121 *vs*. n=133, p=0.35). Digestive tract was the most frequent location of major hemorrhage (31%, n=171) [4]. In the ENGAGE-AF TIMI 48 study, edoxaban 60 mg once daily was associated with increased risk of GI bleeding than warfarin (1.51% *vs*. 1.23% per year, p=0.03), while edoxaban 30 mg once daily reduced the rate of GI bleeding compared with warfarin (0.82% *vs*. 1.23% per year, p<0.001) [7].

The risk of GI bleeding varies among different NOAC regimens. A meta-analysis of phase III randomized controlled trials showed that rivaroxaban and high dosage of dabigatran and edoxaban significantly increased the number of GI bleeding in patients with non-valvular AF [23]. The rate of GI bleeding was similar in patients receiving apixaban, low-dose dabigatran and warfarin. This results suggest that rivaroxaban and high dosage of dabigatran and edoxaban should be avoided in patients at elevated risk of GI bleed.

In a recent network meta-analysis, rivaroxaban, but not apixaban, edoxaban and dabigatran, was associated with increased risk of major GI bleeding [24]. The highest probability of being the safest option had apixaban with regard to the major GI bleeding risk.

Typically, patients treated with warfarin, aspirin or non-steroidal anti-inflammatory drugs bleed from the upper GI tract. Similarly, the most frequent site of major bleeding among patients receiving rivaroxaban in the ROCKET AF trial was the upper GI tract [5]. On the other hand, in the RE-LY trial, the most common location for major bleeding in patients on dabigatran therapy was the lower GI tract [6]. In the ENGAGE-AF TIMI 48 participants, the rate of lower GI bleeding was significantly lower with low-dose edoxaban than with warfarin and the rates of upper GI bleeding were similar in both groups [7]

## AF PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTIONS

Triple antithrombotic therapy (TAT) with OAC plus two antiplatelet agents (aspirin and P2Y12 inhibitor) in patients with AF after an acute coronary syndrome (ACS) or undergoing elective percutaneous coronary intervention (PCI) is associated with a 2- to 4- fold increase in the risk of major bleeding [25]. Clinical decision making in these patients requires the evaluation of patient's stroke and bleeding risks. In this setting, the European Society of Cardiology guidelines recommend the preference of NOACs over VKAs and minimizing the duration of triple antithrombotic therapy (e.g. ≤1 week) [26]. Novel P2Y12 inhibitors (i.e. ticagrelor and prasugrel) should be avoided as a part of TAT. In patients with AF after ACS and/or PCI, absolute bleeding risk may be also reduced by using a radial approach, adding a proton pomp inhibitor to limit GI bleeding, and avoiding the use of non-steroidal anti-inflammatory drugs.

Several recent studies have evaluated the efficacy and safety of double *vs*. triple antithrombotic therapy in patients with AF and ACS or undergoing PCI. In the WOEST (*What is the Optimal Antiplatelet & Anticoagulant Therapy in Patients with Oral Anticoagulation and Coronary Stenting*) trial, the use of clopidogrel alone compared with aspirin plus clopidogrel in the patients on VKA therapy reduced the number of all bleeding events as well as all-cause mortality without any increase in ischemic events [27].

In the ISAR-TRIPLE (*Triple Therapy in Patients on Oral Anticoagulation After Drug Eluting Stent Implantation*) trial, patients receiving concomitant aspirin and VKA were randomized to 6-week clopidogrel therapy or 6-month clopidogrel therapy. There was no significant differences in the combined ischemic endpoint (cardiac death, myocardial infarction, definite stent thrombosis, and ischemic stroke) or in the bleeding endpoint of TIMI major bleeding between the groups [28]. These findings suggests that TAT may be minimized in patients with elevated bleeding risk.

The results of recent randomized controlled trials clearly indicate that the use of NOACs instead of VKAs in AF patients undergoing PCI is associated with a reduction of bleeding risk. In the PIONEER AF-PCI (*A Study Exploring Two Strategies of Rivaroxaban and One of Oral Vitamin K Antagonist in Patients With Atrial Fibrillation Who Undergo Percutaneous Coronary Intervention*) trial, participants undergoing PCI with placement of stents were randomized in a 1:1:1 ratio to rivaroxaban 15 mg plus P2Y12 inhibitor for 12 months, very low-dose rivaroxaban (2.5 mg twice daily) plus DAPT for 1, 6 or 12 months, and standard therapy with a dose-adjusted vitamin K antagonist plus DAPT for 1, 6 or 12 months. All groups had a similar efficacy rates but broad CIs were observed. The administration of low-dose rivaroxaban plus P2Y12 inhibitor for 12 months or very-low-dose rivaroxaban plus DAPT for 1,6 or 12 months reduced the rates of clinically significant bleeding compared with standard VKA plus DAPT for 1, 6, or 12 months therapy [29].

In the REDUAL-PCI (*Evaluation of Dual Therapy With Dabigatran vs. Triple Therapy With Warfarin in Patients With AF That Undergo a PCI With Stenting*) trial, dabigatran 150 mg twice daily or 110 mg twice daily plus P2Y12 inhibitor *vs*. standard TAT was associated with significantly lower rate of major or clinically relevant non-major bleeding among AF patients after PCI [30]. A numerical increase in MI and definite stent thrombosis in the dual therapy dabigatran 110 mg group was observed. Adverse cardiac events were similar between study groups. The AUGUSTUS (*An Open-label, 2 Factorial, Randomized Controlled, Clinical Trial to Evaluate the Safety of Apixaban vs. Vitamin K Antagonist and Aspirin vs. Aspirin Placebo in Patients With Atrial Fibrillation and Acute Coronary Syndrome or Percutaneous Coronary Intervention)* investigators found that an antithrombotic regimen that included apixaban without aspirin among patients with AF and a recent ACS or PCI treated with P2Y12 was associated

**Table 2.** Available reversal agents and suggested use ([37] – modified).

| Reversal Agent | Factor IIa inhibitor (dabigatran) | Factor Xa inhibitors (apixaban, edoxaban, rivaroxaban) |
|---|---|---|
| 4F-PCC | second line | first line |
| aPCC | second line | second line |
| Idarucizumab | first line | not indicated |
| Andexanet alfa | not indicated | first line |
| Plasma | not indicated | not indicated |

Note: aPCC – activated prothrombin complex concentrate; 4F-PCC – the 4-factor prothrombin complex concentrate

with lower rate of bleeding and hospitalizations without significant differences in the number of ischemic events compared with VKA, aspirin or both therapy [31]. In AF patients undergoing PCI in the ENTRUST-AF PCI trial, the edoxaban-based regimen (edoxaban 60 mg daily plus P2Y12 inhibitor) was non-inferior in terms of bleeding events compared with VKA in combination with a P2Y12 inhibitor and aspirin, without significant differences in the rates of ischemic events [32].

## SAFETY *VS*. EFFICACY

Anticoagulation therapy should be administrated carefully and individually. Indirect comparisons and network meta-analyses showed generally similar efficacy and differences in safety profiles of NOACs [33]. In the ARISTOPHANES *(Anticoagulants for Reduction in Stroke: Observational Pooled Analysis on Health Outcomes and Experience of Patients)* study, apixaban was superior to warfarin in preventing stroke/systemic embolism, major bleeding and intracranial hemorrhage. Rivaroxaban was associated with significantly lower risk of stroke/systemic embolism and higher rate of major bleeding compared with warfarin [34].

In clinical practice, decision to prescribe standard or reduced dose of NOACs should be made of the basis of specific considerations (age, renal function and use of concomitant medications). However, some data suggest that a substantial number of patients are receiving reduced dose of NOACs without fulfillment Food and Drug Administration (FDA) dosing recommendation for such dosing. Inappropriate dosing of NOAC may have important clinical implications for treatment effectiveness.

In a propensity weighted nationwide study of reduced doses of NOAC, apixaban 2.5 mg twice daily was related with a trend towards higher risk of ischemic stroke/systemic embolism compared with warfarin [35]. In the ORBIT-AF II (*Outcomes Registry for Better Informed Treatment of Atrial Fibrillation II*) registry, a reduced NOAC dose was prescribed to 16% patients which was consistent with FDA labeling in 43% cases. Moreover, patients receiving inappropriately reduced-dose NOACs had higher rates of thromboembolic events (2.11 versus 1.35 events per 100 patient years; HR 1.56; 95% CI 0.92-2.67) and death (6.77 versus 2.60; HR 2.61; 95% CI 1.86-3.67) compared with individuals appropriate receiving standard dosing [36].

## MANAGEMENT OF NOAC-RELATED BLEEDING

Currently, we dispose limited therapeutic options in patients with bleeding during NOAC therapy (Table 2) [3, 37]. An obligate first step in the initial evaluation of such individuals is to determine hemodynamic status, blood pressure, coagulation parameters, blood count and renal function. In cases of minor hemorrhage mechanical compression or minor surgery ought to be sufficient. Delay NOAC for one dose or one day should be considered. Due to NOACs short half-life time, after 12-24 hours after the last dose we may expect improvement of the coagulation.

Management of moderate to severe bleeding may include fluid replacement, blood transfusion and a procedure to control bleeding (e.g. endoscopy). Oral activated charcoal can be used if NOAC was recently ingested. Owing to low protein binding, hemodialysis reduces the plasma concentration of dabigatran.

In a life-threatening, critical site bleed, or in situations in which bleeding cannot be controlled, reversal of NOACs is indicated. The rational use of antidotes is crucial. Relative short half-times of NOACs reduce the need for use of an antidote in clinical practice.

Administration of prothrombin complex concentrates (PCC) should be consider for NOAC related bleeding if specific reversal agents are not available.

Idarucizumab is a humanized monoclonal antibody fragment designed to reversal the effect of dabigatran. Idarucizumab and dabigatran form complexes cleared by the kidneys. In the RE-VERSE (A Study of the RE-VERSal Effects of Idarucizumab on Active Dabigatran) AD study, in over 500 patients intravenous infusion of 5 g of idarucizumab rapidly, durably and safely reversed the anticoagulant effect of dabigatran in patients who had uncontrolled bleeding or were about to undergo an urgent major procedures [38]. Idarucizumab is widely available almost all over the world. Andexanet alfa is a recombinant modified factor Xa molecule for reversal of factor Xa inhibitors. This first and only available antidote was approved by the Food and Drug Administration in patients treated with rivaroxaban or apixaban when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding. Andexanet alfa acts as a decoy of Xa, that binds factor Xa inhibitors without enzymatic activity. In the ANNEXA-4 study, andexanet alfa was administrated as a bolus injection, followed by a 2-hour infusion. In 352 patients with acute major bleeding within 18 hours after receiving apixaban, rivaroxaban, edoxaban at any dose or enoxaparin at a dose of at least 1 mg per kilogram of

body weight per day, treatment with andexanet alfa substantially reduced anty-Xa activity. Most of patients (82%) had excellent or good hemostatic efficacy [39]. Unfortunately, there is still a limited access to andexanet alfa.

Ciraparantag (PER977) is a small synthetic molecule that binds heparin and the oral direct factor Xa (FXa) and factor IIa (FIIa) inhibitors. Ciraparantag in healthy subjects was safe and completely reversed the anticoagulant effects of edoxaban [40]. Unfortunately, this universal antidote has not been studied in major bleeding or patients who needed urgent surgery.

A clinical concern of NOACs antidotes is anticoagulation rebound (reappearance of NOAC anticoagulation activity after reversal). In the RE-VERSE AD study, reappearance of levels above 20 ng per milliliter of dabigatran was observed in 23% patients (n=114), mainly after 12 hours, and was associated with continued or recurrent bleeding in 20 patients. Three patients required administration of additional dose of idarucizumab [38]. In the ANNEXA-4 (*Prospective, Open-Label Study of Andexanet Alfa in Patients Receiving a Factor Xa Inhibitor Who Have Acute Major Bleeding)* study, rivaroxaban level above 50 ng/mL was documented 4 hours after andexanet alfa infusion. Further studies are needed to determinate the role played by anticoagulation rebound in the clinical practice [39].

## CONCLUSIONS

Scientific evidence from both randomized clinical trials and registries indicates favorable risk to benefit profile of NOACs compared with warfarin in patients with non-valvular AF. Substantial benefits of NOACs include: a reduced risk of ICH, faster onset and offset of action and fewer drug and food interactions. In randomized clinical trials, dabigatran 110 mg, apixaban, and edoxaban have been associated with lower risk of major bleeding compared with VKA. The risk of major bleeding was similar with warfarin for patients taking 150 mg dabigatran or rivaroxaban. NOACs except apixaban and low-dose dabigatran may increase the risk of gastrointestinal bleeding. Visible hematuria during NOAC therapy should be a red flag and underlines the importance of upper urinary tract imaging and cystoscopy to exclude neoplasia. Management of bleeding in anticoagulated patients who experience major bleeding, trauma or undergoing emergency surgery may require antidote administration.

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## Conflict of interest

*Anna Tomaszuk-Kazberuk – lectures and consultations for Boehringer–Ingelheim and consultation for Bayer.*
*Marek Koziński – received speaker fees from Bayer, Boehringer–Ingelheim and Pfizer*

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REVIEW ARTICLE

# INFORMATION AND COMMUNICATION TECHNOLOGIES IN PUBLIC MANAGEMENT OF THE HEALTHCARE INSTITUTIONS NETWORK DURING COVID-19 PANDEMICS

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## ABSTRACT

**The aim** was to study the structure, routes and algorithms of telemedical network in Odessa region and its impact on the spread of COVID-19 pandemics.

**Materials and methods:** We have analysed the data of the official reporting documentation provided by the Department of Healthcare of Odessa region. So the description of legislative acts and structure of telmedical network of Odessa region is given. In more detail, we described functions and capabilities of telmedical network COVID-19 pandemics.

**Conclusions:** Studies have shown that Telemedicine can be used in 5 main directions: Triage and decisions regarding hospitalization to hospitals designed for COVID-19 patients or to other hospitals. Outpatient consulting in COVID-19 patients who stay at home. Coordination of activities of different hospitals; provision of specialized consulting. Training of healthcare providers for counter-epidemic measures and management of COVID-19 patients. Routine consulting in patients with chronic disease and in palliative cases. In all this activities significant benefits were founded. But amount of telemedical consultations are quiet low.

As in all viral pandemies remote consultations of patients leeds to lowering in amount of infection of COVID-19 patients.

**KEY WORDS:** COVID-19, Triage, Telemedicine, Remote Consultation, Health Communication

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## INTRODUCTION

Humanity faces a new pandemic of SARS-CoV-2 infection which was declared as an international public health emergency by WHO. Fast and uncontrolled spread of COVID-19 infection poses a serious threat to the health of the entire population. The most recent pandemic of influenza A (H1N1) virus occurred in 2009. Just for first year it caused between 100 000 and 400 000 deaths globally. But it was not so massive as a current COVID-19 pandemics [1]. According to actual report of Kyiv school of economics on 1 of August it is already 17 106 007 infected and 668 910 death in the world. The problem also concerns Ukraine and total score of infected and death are 71056 and 1709 (2,4%) respectively [2,3].

Healthcare systems of multiple countries turned to be totally unfit during the epidemics: healthcare providers were not only psychologically unready to face the unusual workload pressure but also unaware of crucial counter-epidemic actions; moreover, many of them were unable to use personal protection items [4].

Therefore, many healthcare professionals have fallen victims to COVID-19 infection themselves. The burden of COVID-19 among healthcare professionals at least from 23 000 to 9000 are infected and hundreds have already died [5]. But governments do not collect the data accurately, so the total score is unknown. In Ukraine 8 745 healthcare professionals were infected and 43 died because of complications according to information from Ministry of Health. The well-being of the health care workforce is the cornerstone of every well-functioning health system [6,7].

Many scientists report failure of old strategy of coordination of healthcare institutions and other contributors [8]. The new strategy should include changed approaches to diagnosing, treatment and development of drugs and vaccines. Actions that can overcome the epidemics include changes of communication activities targeting maximum isolation of infected persons and contacts as well as social distancing of healthy persons. Another significant contribution is coordination provided by administrative institutions. Many authors consider information and communication technologies a central element of counter-epidemics strategy [9,11].

## THE AIM

Description of structure, routes and algorithms of telemedical network in Odessa region as a central communication component of healthcare institutions management under the conditions of COVID-19 pandemics.

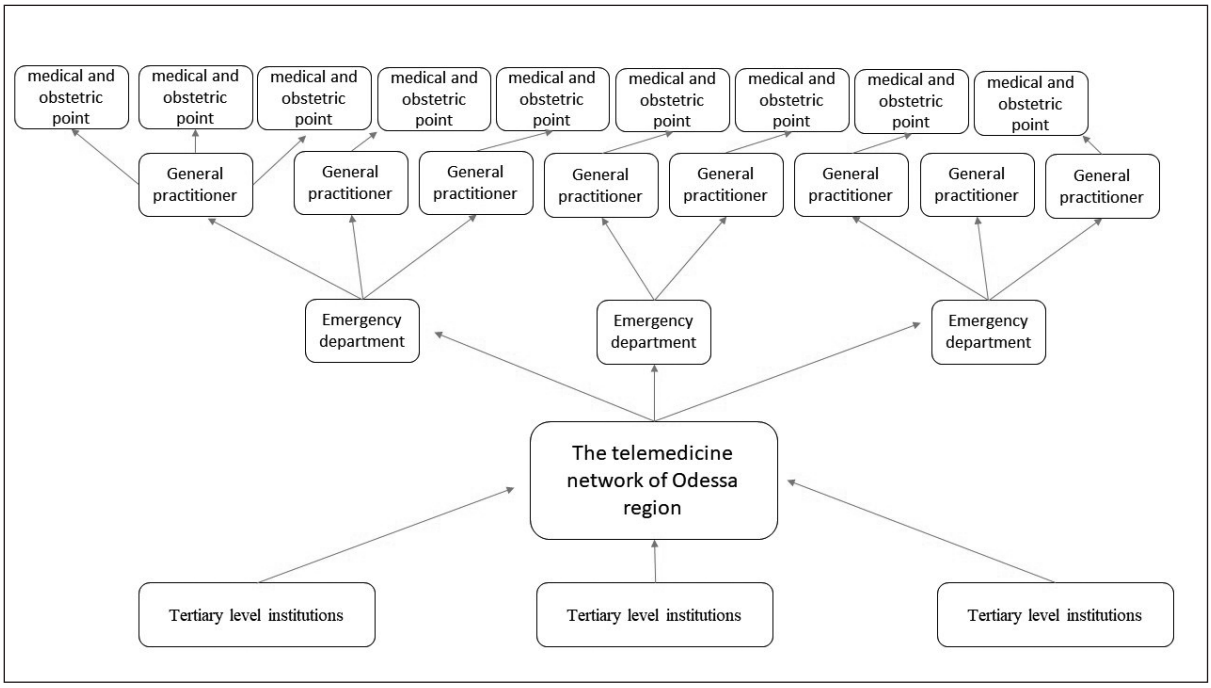


**Fig. 1.** The structure of the telemedicine network of Odessa region

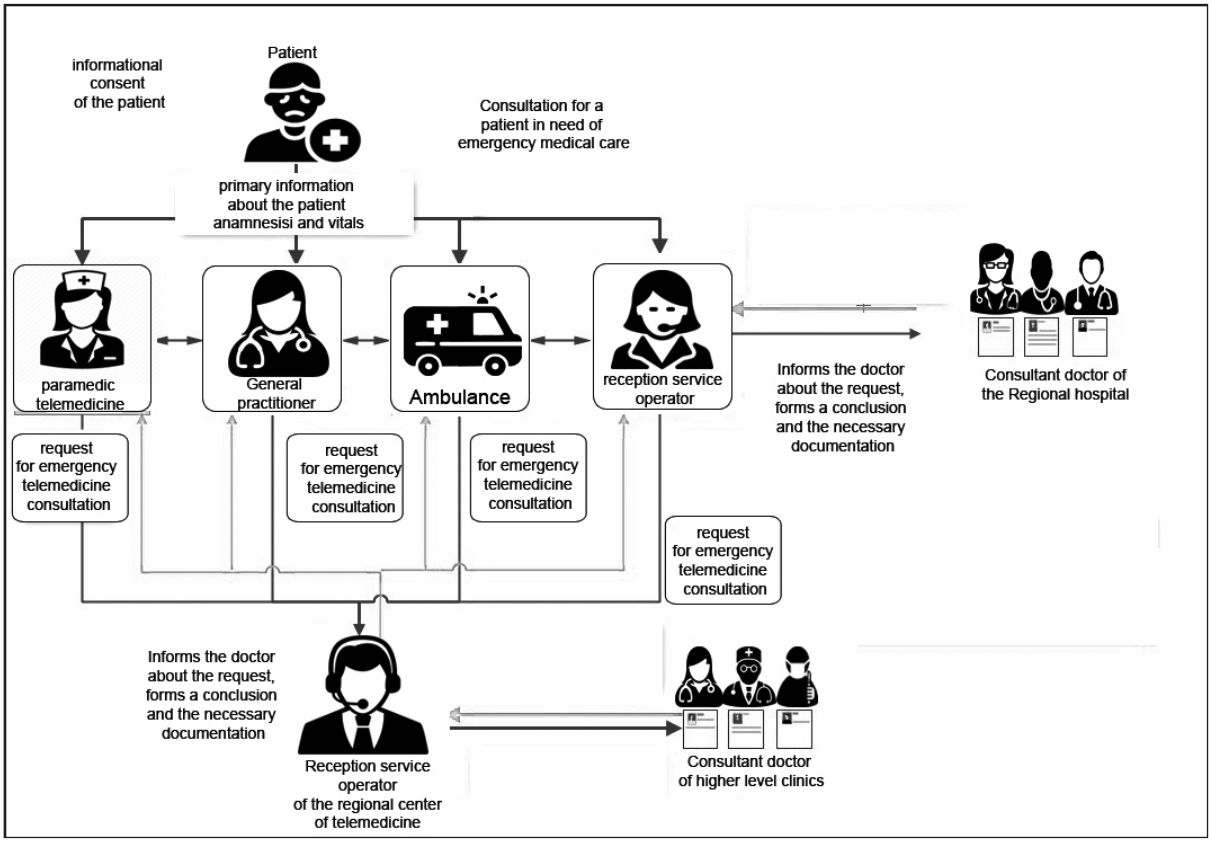


**Fig. 2.** Technological scheme of emergency telemedicine consultations of a patient with suspected coronavirus is designed to work for a limited period of time.

Review of healthcare institutions management practices under the conditions of COVID-19 pandemics including the use of information and communication technologies both in Odessa region and in developed countries. Analyze the telemedicine system coordination capacity in Odessa region in response to COVID-19 pandemics based on published data.

## MATERIALS AND METHODS

This is a descriptive study providing empirical information

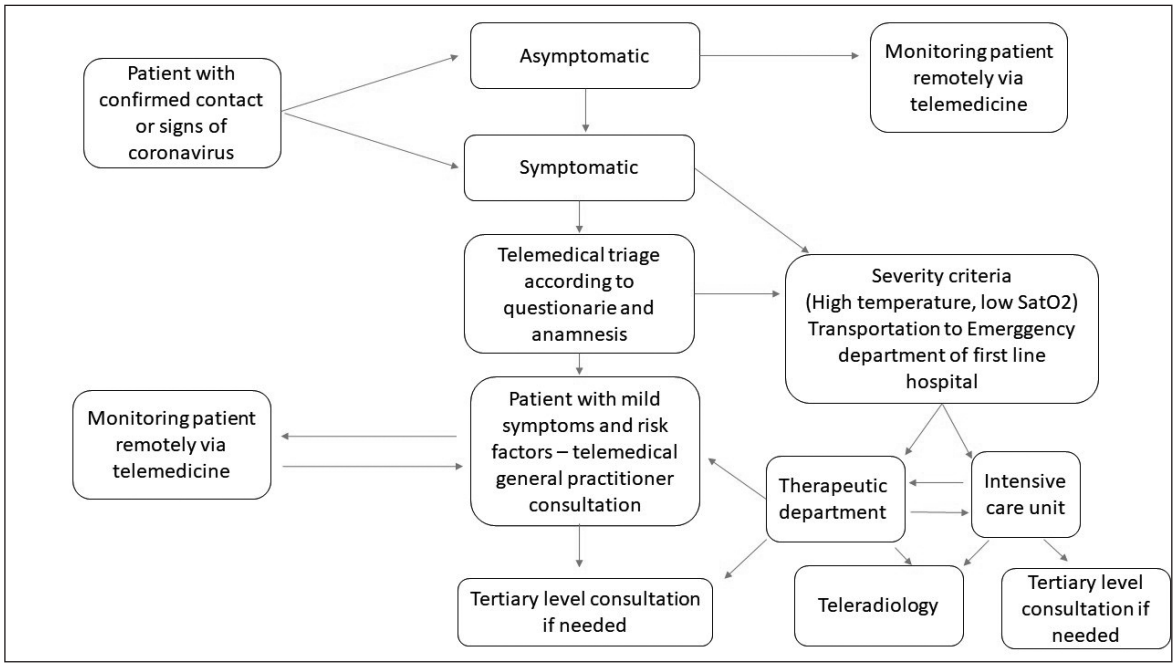


**Fig. 3** Telemedical triage of patient with COVID-19

**Table I.** Distribution of patients with coronavirus according to age groups.

| Age intervals | Patients n = 65 |
|---|---|
| 0-1 years | 0 |
| 1-14 years | 0 |
| 14-40 years | 16 |
| 40-60 years | 30 |
| Above 60 years | 19 |

**Table II.** Detected contact with COVID-19 among the consulted patients.

| Risk factors | Patients n = 65 | % |
|---|---|---|
| Detected contact with COVID-19 | 10 | 15,38% |
| Trips abroad 14 days before the first symptoms | 2 | 3,07% |
| No detected contact or trips abroad 14 days before the first symptoms | 53 | 81,53% |

and descriptive statistics of public management system regarding the region-based pattern of COVID-19 patients hospitalization in healthcare institutions of Odessa region supported by telemedicine network (Telehealth system) with Odessa Regional Telemedicine Center as the leading element of patient's routes coordination.

The Odessa region telemedicine network has been developed in compliance with the following legislation acts: the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Health Care", the Law of Ukraine "Improvement of medical care accessibility and quality in rural areas", the Law of Ukraine "Governmental financial guarantees of medical care", the Order of Ministry of Health (MoH) of Ukraine dated 28.09.2012, №752 "Order of medical care quality control", the Order of MoH of Ukraine dated 21.09.2012, № 732 "Approval of the Plan of MoH of Ukraine regarding the implementation of medical care quality management program in Ukraine until 2020", the Order of MoH of Ukraine dated 19.10.2015, № 681 "Approval of specifications for implementation of telemedicine in the field of healthcare", as well as the Orders of Odessa region Healthcare department "Organization of medical care based on telemedicine technologies in the Odessa region" and "Organization of urgent outpatient medical care based on telemedicine technologies in the Odessa region".

The telemedicine network of Odessa region (Figure 1) includes 89 healthcare institutions.

- 29 primary level medical care institutions;
- 37 secondary level institutions;
- 19 tertiary level institutions;

The network is subdivided in two tiers: 32 telemedicine offices in regional healthcare institutions are running the consulting projects; they are connected to 37 offices and 29 telemedicine checkpoints that are receiving the consulting info.

Regional center of telemedicine is the central coordination element of the system. It runs the management, organization and technical support and provides methodology framework for the system. The center includes the following elements:

- Operative reception service, 7 positions;
- Technical maintenance department, 5 positions;
- Information support department, 5 positions.

Moreover, 18 reception service operators are localized in the telemedicine offices at the regional hospitals, namely: the Regional hospital of Sarata, the Regional hospital of Kodyma, the Regional hospital of Podilsk, the Regional

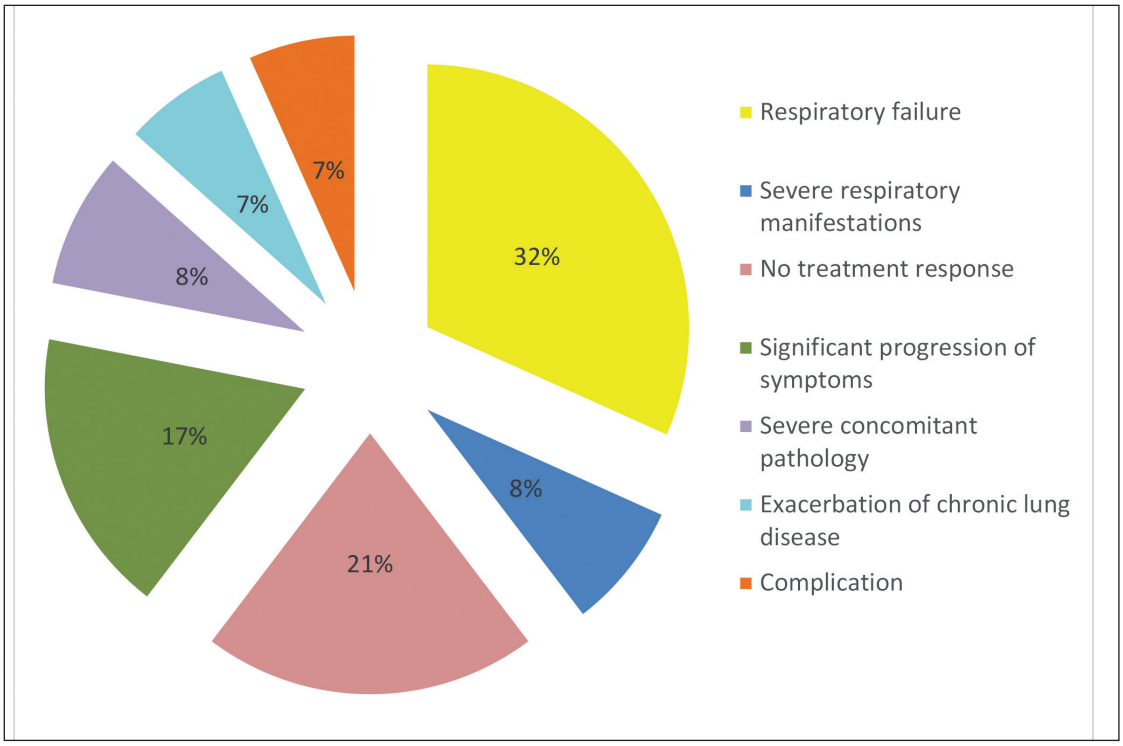


**Fig. 4.** Causes for hospitalization of patients with acute respiratory disease COVID-19, after telemedicine consultation.

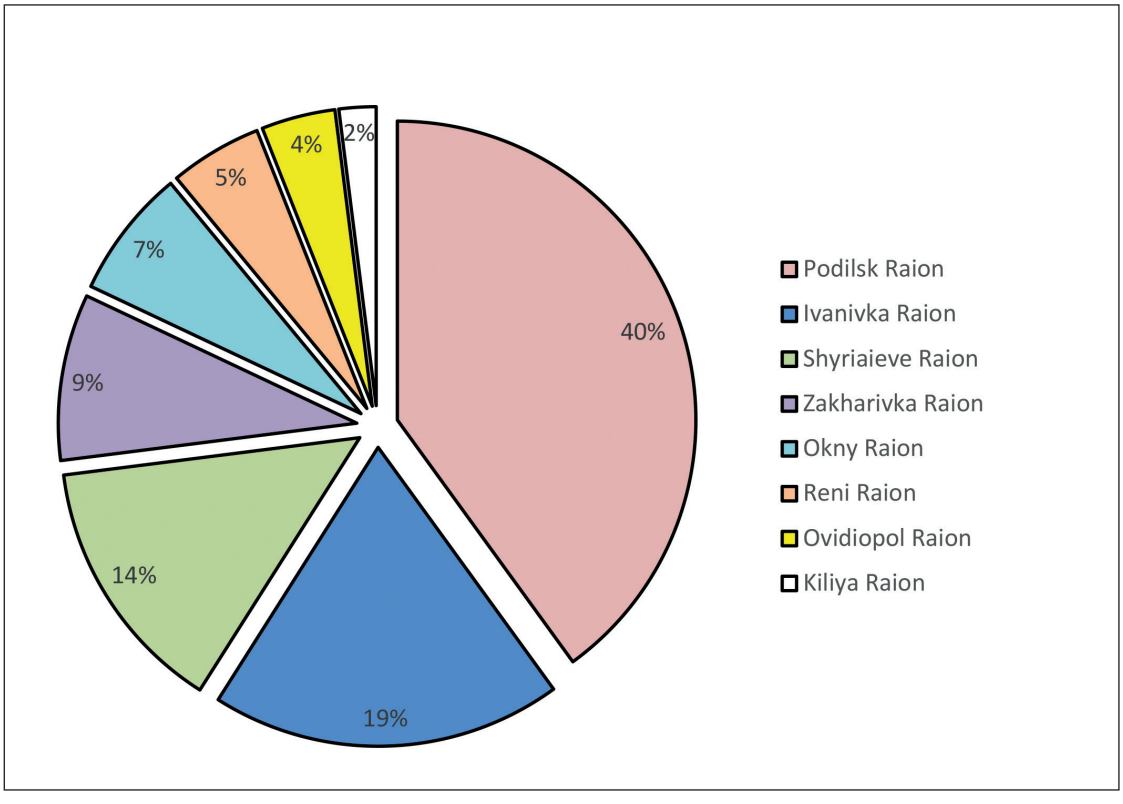


**Fig. 5.** Distribution of telemedical consultations by raions of Odessa oblast.

hospital of Ovidiopol, the Regional hospital of Velyka Mykhailivka, the Regional hospital of Zakharivka, the Regional hospital of Lybashivka, the Regional hospital of Tarutino, the Regional hospital of Izmail, the Regional hospital of Mykolaivka, the Regional hospital of Teplodar, etc.

The regional center of telemedicine is the platform for information and medical events. It includes the following services:

- Medical information system;
- Telemedicine consulting;
- Education;
- Technical maintenance;

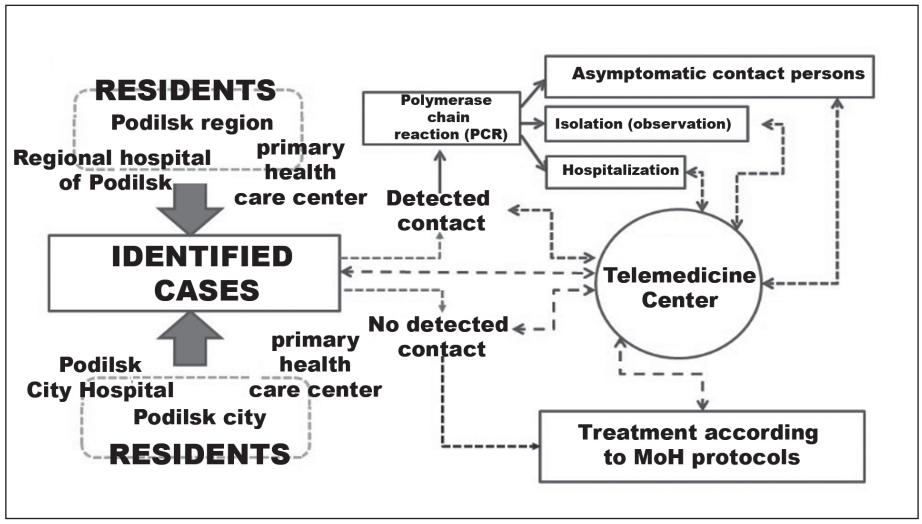


**Fig. 6.** Algorithm for monitoring the situation in the Podolsk raion

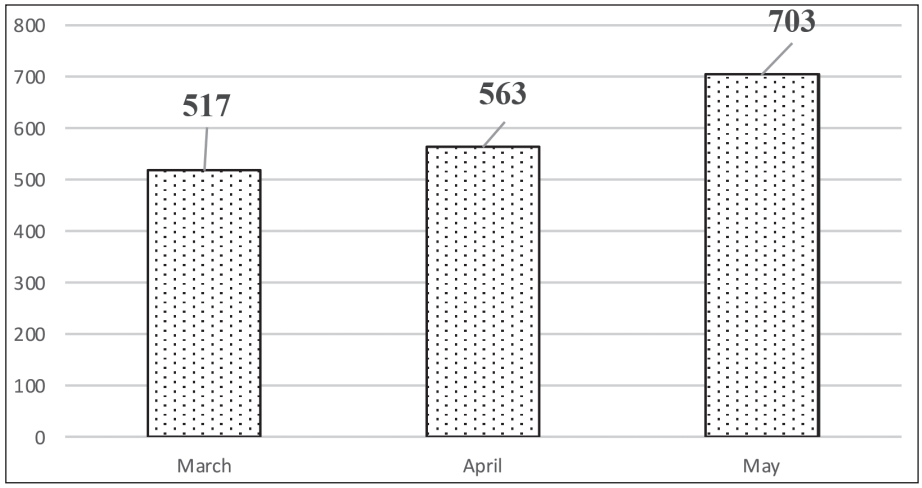


**Fig. 7.** Telemedicine consultations for non – COVID-19 patients during the quarantine period (March – May)

- Archiving of investigations and laboratory studies results;
- Organization of mobile diagnosing teams.

The regional information medical system with a telemedicine domain is located in the cloud-based protected service. This system joins the existing medical tools into a single program interface. The system also includes tools for medical documents turnover, medical documents and consulting video records archiving, as well as the server holding the imaging medical records.

Consulting in urgent cases is the basic function of telemedicine network. In 2019 the system has run 463 urgent consultations; in 290 cases the issues were managed through remote consulting only without a visit of a consulting specialist. In 91 cases outpatient consultations were conducted. In 82 cases patients were transported to healthcare institutions with an escorting anesthesiologist.

At the beginning of COVID-19 epidemics in compliance with the Order No395 of Odessa regional administration "Approval of regional algorithm for coronavirus patients (COVID 19)" the structure of telemedicine network at Odessa regional center of telemedicine has been changed. The network has been re-arranged in order to provide the interactions between all elements of medical care system and to connect other structures, like managing institutions and healthcare institutions running the counter-epidemics actions. The functions of the network include implementation of actions targeting better public access to medical care during the COVID-19 quarantine in Odessa region. The system is design to implement the principle of single medical information space during the COVID-19 quarantine. Provision of common approaches and criteria for healthcare system telemedicine technologies application and evaluation, as well as establishment of possible use and development of telemedicine system during COVID-19 quarantine are also targeted by the system updates.

The algorithm of consultation for patients with suspected coronavirus infection has been outlined (Figure 2).

The new pattern includes the following elements:

· Expanded urinalysis
· Standard urinalysis
· Sputum analysis; double sputum analysis
· Chest X-ray – 2 or 3 images
· Chest CT with intravenous contrast (if required)
· Blood analysis (urea and creatinine)
· O2 saturation level
· US examination of pleural cavities
· ECG

Urgent consultations were conducted in patients with suspected coronavirus in compliance with the approved algorithm.

Medical triage with route establishment is an important element of telemedicine. The template of-telemedicine triage is outlined in Figure 3.

Two types of patients are outlined in the telemedicine triage system: patients with coronavirus infection manifestations and contact persons. These patients are subject to primary telemedicine examination. Symptoms-free patients and patients with mild disease are sent for isolation containment under supervision of the family doctor. The supervising doctor contacts the patient routinely for status monitoring. Mobile medical team can be sent to the patient. These teams are designed to respond the challenges of acute COVID-19.

If high probability of acute COVID-19 is found during the primary remote examination in patients with moderate to severe condition these patients are hospitalized by the specialized emergency ambulance teams to pre-established hospitals designed for appropriate cases.

Telemedicine is also used for training of healthcare professionals. Patterns of patients with suspected coronavirus infection are reviewed during these trainings.

Experts from Healthcare Department, regional and municipal hospitals contributed to trainings that enrolled healthcare institutions management stuff and their subordinates. Patterns of response to COVID-19 cases and patient's route planning are reviewed in compliance with effective normative acts. Special attention is paid to individual protection items use and utilization, possible actions at the suspected infection site, COVID-19 treatment protocols, smear sampling, and resuscitation protocols in COVID-19 patients. Telemedicine training protocols for healthcare institutions seniors and subordinates were developed in compliance with Dreyfus model of skill acquisition.

## REVIEW

During the study period (March 1 – June 1, 2020) totally 164 consultations for COVID-19 cases were conducted in 65 patients. 14 healthcare providers were running the consulting.

Note, that the total number of patients with suspected COVID-19 included 18 healthcare professionals and 47 other patients.

During the 3 months' period the number of urgent consultations increased by 13% compared to similar period prior to quarantine resulting in respective increase in the number of consulting specialists. Specialists in X-ray and pulmonologists experienced the highest burden (5% and 16% respectively).

Table I shows that consultations were most common in patients from 40-65 years' age group with suspected COVID-19 infection.

Also many consultations were reported in patients returning from other countries during the first 14 days prior to infection manifestations (Table II).

Table III lists the reported symptoms.

Figure 4 shows the details regarding remote triage and algorithm of decision making regarding the hospitalization. Hospitalization was most common in patients with respiratory failure (52 patients). Also patients with no treatment response (34) and rapid disease progression (29) were hospitalized.

The distribution of consultations in various districts of Odessa region was uneven.

Figure 5 shows the highest number of consultations in Podilsk raion due to disease outbreak and the respective decision of the Comission on technogenic and environmental safety and emergencies regarding the enforcement of quarantine measures in the respective area. Note the highest number of healthcare providers with COVID-19 infection reported in Podilsk raion. Therefore, it has been decided to use telemedicine as the basic tool for coordination and consulting of suspected infection cases and for other patients as well.

Use of telemedicine resulted in tailored response to challenges including shifting of patient's routes. The respective case description algorithm has been proposed (Figure 6).

Figure 7 demonstrates higher number of consultations in patients with chronic diseases as well as in complex cases and for palliative reasons. These consultations were justified by patient's safety issues and complex multi-link logistics associated with closure of bus and railway connections in Odessa region during the quarantine. Figure 7 demonstrates the 150% growth of consultations number.

## DISCUSSION

Experience of other countries points to hospital network overload as the main challenge during COVID-19 epidemics resulting in high level of fatal outcomes both in COVID-19 patients and in other patients with critical conditions who failed to receive timely medical care [12;13]. Many authors report high capacity of telemedicine network as a powerful tool of healthcare institutions coordination in response to COVID-19 patient's overflow [10.11.14]. 5 main directions of telemedicine activities are summarized below:

1. Triage and decisions regarding hospitalization to hospitals designed for COVID-19 patients or to other hospitals.
2. Outpatient consulting in COVID-19 patients who stay at home.
3. Coordination of activities of different hospitals; provision of specialized consulting.
4. Training of healthcare providers for counter-epidemic measures and management of COVID-19 patients.
5. Routine consulting in patients with chronic disease and in palliative cases.

Therefore, organization of telemedicine network in Odessa region is generally compliant with modern trends.

The system of remote consulting in Odessa region was targeted to minimize the time needed for provision of specialized consultations. Most of consultations were focused on analysis of investigations, X-rays, CT imaging, etc.

Application of standardized algorithms enabled minimization of infection transmission both for healthcare providers

**Table III.** Classification of patients with COVID-19 according to symptom

| Symptom | Patients n = 65 | % |
|---|---|---|
| Cough | 65 | 100% |
| Shortness of breath or difficulty breathing | 33 | 50,76% |
| Respiratory insufficiency | 33 | 50,76% |
| Chest x-ray findings | 65 | 100% |
| Vomiting | 6 | 9,23% |
| Diarrhea | 5 | 7,69% |
| Nausea | 6 | 9,23% |
| Abdominal discomfort | 6 | 9,23% |
| Fever or chills | 28 | 43,07% |
| Congestion or runny nose | 15 | 23,07% |
| Sore throat | 49 | 75,38% |
| New loss of taste or smell | 17 | 26,15% |
| Headache | 65 | 100% |
| Muscle or body aches | 53 | 81,43% |
| Fatigue | 40 | 61,53% |

and patients. Use of telemedicine for consulting and triage was crucial during the outbreak in Podilsk city. The clear roadmap has been developed showing possible routes for patients with disease manifestations. The actual hospitalization route depended on groups of reported symptoms.

Remote training of healthcare seniors and providers resulted in beneficial outcomes also. In compliance with Dreifus brothers model, the training levels were Novice and Advanced beginner [15,16]; therefore, high levels of mistakes requiring correction were reported. The trainees were inexperienced enough to fail during dressing in protective clothing. Respectively, trainings were focused on context independent rules and algorithm (if X do Y format). Multiple practical workouts were conducted. The mistakes of trainees were noticed and corrected. After the correction of mistakes, the instructing experts were running the evaluation of trainees.

Telemedicine enabled training of high number of healthcare providers in short terms without a need for experts to travel to training sites. It contributed to lower infection risk and resulted in lower expenses compared to standard trainings in the healthcare network of Odessa region.

Survey results (no statistical analysis conducted) demonstrated high satisfaction with telemedicine system both in COVID-19 patients and in other patients. The results remained promising despite the rapid increase of consultations number and involvement of experts with no prior experience of remote consulting.

However, certain issues are reported in the telemedicine network of Odessa regioni:

First, the low coverage of patients should be noted. The total number of suspected cases was 2210 with 886 infected patients (as of June 1); however, only 65 of them received remote consulting (7.3%). However, it cannot be stated that such result was unexpected. This condition is associated with clinical route of medical care designed for COVID-19 patients. Specialized emergency ambulance teams were directed to patients reporting high temperature and signs of respiratory infection. These ambulances were delivering patients to pre-established medical institutions designed for COVID-19 cases.

It's a pity, but ambulance teams were practically neglecting the telemedicine triage system. However, use of this system could have resulted in lower patient's loading of emergency departments.

Family doctors also failed to use the monitoring capacities. In patients reporting disease aggravation family doctors were recommending emergency aid supported by ambulance teams not equipped with telemedicine tools. Inclusion of personal telemedicine tools into the ambulance kits could improve the capacity for consulting in patients who remain at home.

Development of remote consulting scenarios could help to monitor the patients during isolation period: monitoring for patients with symptoms-free disease, specialized consulting for patients with mild disease, medical service for treatment of patients who are unable to reach specialized medical care and even geo-location monitoring for persons who are obliged to remain isolated after having returned from countries with COVID-19 outbreaks.

## CONCLUSIONS

Application of information and communication technologies as the core component has contributed to routing of COVID-19 patients resulting in lower infection level both among patients and healthcare providers.

Involvement of emergency care service to telemedicine triage network in for COVID-19 patients can result in lower burden for emergency departments.

Further development of telemedicine network requires time, resources, specialists and respective normative acts. Application of telemedicine network in Odessa region is an example of successful use of information and communication technologies for public management of healthcare institutions network in response to COVID-19 pandemics. This experience can be shared with other regions of Ukraine.

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REVIEW ARTICLE

# RHABDOMYOLYSIS – INDUCED ACUTE KIDNEY INJURY – AN UNDERESTIMATED PROBLEM

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**ABSTRACT**

Rhabdomyolysis is a condition characterized by muscle damage and the release of intracellular muscle contents into the circulation. It leads to a lot of complications e.g. hyperkalemia, hyperphosphatemia, and calcium disorders. The etiology is multifactorial. Severity ranges from mildly muscle weakness without any systemic complications, to life-threatening multi-organ damage. The most common and serious systemic complication is acute kidney injury (AKI). In the review, we address the epidemiology, causes, and treatment. The ideal would be to predict and prevent rhabdomyolysis at all, but when it is impossible, the key to successful treatment is its rapid implementation. Therapy should be selected individually, adapting to the triggers, and closely monitoring the patient's condition. Early implementation of fluid therapy appears to be crucial. Electrolyte disturbances should always be detected in the early stages and carefully treated. The use of bicarbonates or diuretics may also be helpful, but especially in the latter case, the indications should be well evaluated, remembering to avoid hypovolemia. Renal replacement therapy is often implemented due to water-electrolyte or acid-base disorders. Proper diagnosis and early therapy implementation improve patient outcomes, in particular in the face of new infectious dangers and global underestimating of the disease.

**KEY WORDS:** rhabdomyolysis, acute kidney injury, myoglobinuria, cytokine adsorber

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## INTRODUCTION

Rhabdomyolysis (RM) is a common, but underestimated disease, which can be caused by many different reasons. The best known is a crush syndrome – extensive muscle damage after direct injury, trauma, or compression, very often accompanying natural and manmade disasters e.g. earthquakes, warfare, or everyday accidents. This clinical entity was first described in 1941 when Bywaters and his team were analyzing patients with renal failure and muscle necrosis after a bomb attack, in which many people were buried for several hours [1]. They discovered, in post-mortem examination, the brown pigment in renal tubules – later identified as myoglobin.

## THE AIM

We aimed to address the epidemiology, causes, and treatment, including renal replacement therapy.

## REVIEW AND DISCUSSION

### EPIDEMIOLOGY AND ETIOLOGY

Global rhabdomyolysis mortality is wide – estimated between 2 and 46% and depending on the type of cause, early treatment administration, and the additional comorbid conditions and complications [2]. RM-associated AKI (RM-AKI) frequency has been reported from 17-35% and even to 50% in adults in some studies. In critically sick patients, mortality rates are up to 59% [7-8]. Luckily, most patients recovered and normalized kidney function in a few months [3] but RM-AKI promotes structural changes e.g glomerulosclerosis and significantly increasing the risk of chronic kidney disease (CKD) in long-term observations.

Because of the complexity of influencing factors, there are many risk groups. The most exposed are morbidly obese patients, chronic users of lipid-lowering drugs, post-operative patients, prolonged and alcohol abusers. Some studies show a higher risk in man vs woman, in African-Americans, patients >60 (and <10) years old [9-10]. Significant factors may be more, but there is still a problem of insufficient detection. There are several ways to categorize causes of rhabdomyolysis e.g.:

- physical / non-physical,
- acquired/inherited.

Regardless of the divisions, the path of the muscle destructions and consequences run similarly.

### MECHANISMS

There are a few principle mechanisms of kidney injury. Knowledge of these mechanisms has a key role in understanding the whole process and implementing proper treatment.

#### *OXYDATIVE STRESS*

After muscle damage, intracellular fluid is leaked and then sequestrated in extracellular spaces. This causes decreasing

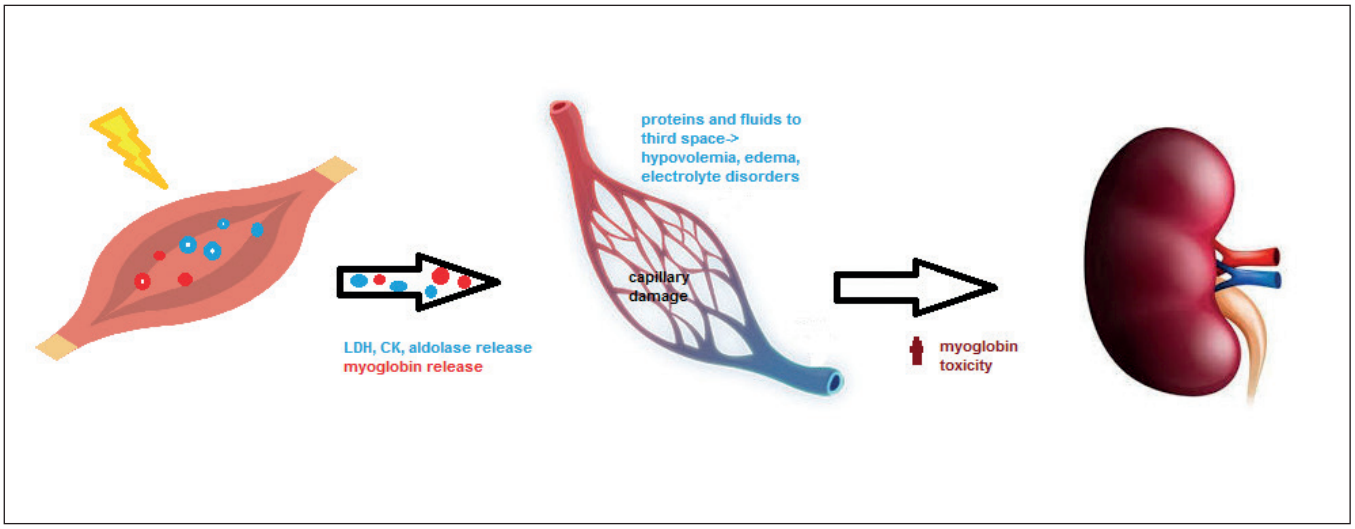


**Fig. 1.** LDH – lactate dehydrogenase, CK – creatine kinase, aldolase – muscle enzymes with myoglobin release into circulation cause capillary damage and by permeability water, proteins and other substances to the third space, increasing kidney damage by hypovolemia and higher myoglobin toxicity

intravascular volume which gives the signal to activate the renin-angiotensin-aldosterone system (RAAS) and afterward reduce renal blood flow. Increased myoglobin level and its components exert a destructive, cytotoxic effect on the nephron – free iron reacts with peroxide compounds generating reactive oxygen species (ROS) [12]. As we well know, ROS infringes renal tubular integrity. At the same time, other muscle components are releasing into the circulation system – uric acid – which forming deposits of crystals and contributing intratubular impediment.

The second mechanism is lipid membrane components peroxidation- after reacting with a ferryl form of myoglobin (redox-cycling) they are entailing metabolic acidosis which intensifies myoglobin toxicity [5, 12]. This mechanism has become the basis for the use of urine alkalization as therapeutic prevention, as we will discuss in another section.

The diagram below in a simplifying way shows the mechanism of kidney damage (Fig. 1).

### *INFLAMMATION*

In the rhabdomyolysis process, damaged muscle cells release a lot of molecules that activating the immune system – among others uric acid, microRNAs, or ligand high mobility group box proteins (HMGB1). These molecules activate dendritic cells, T-lymphocytes, macrophages, Toll-like receptors (TLRs), and nuclear factor kappa beta (NF-κβ). According to this, we have high production of proinflammatory cytokines such as a tumor necrosis factor-alpha (TNF-alpha) [15-17]. Another molecule effects inflammatory reaction in endothelium and the tubular epithelium is myoglobin-derived heme. Exposed cells increasing expression adhesive molecules such as ICAM-1 or VCAM-1 and afterward strengthen the inflammatory response. In consequence, we have the proliferating, pro-fibrotic path, and in the aftermath – glomerulosclerosis.

### *INTRATUBULAR OBSTRUCTION AND APOPTOSIS*

Characteristic manifestation in the tubular lumen is pigment deposits – after muscle cell disintegration, myoglobin and the Tamm-Horsfall protein, precipitate and forming tubular casts. Volume depletion and acidic urine Ph promoting this process. Some authors say that aside from preventing apoptosis there is no specific intervention required [12].

### *VASOCONSTRICTION*

Most of the above have one combined effect – vasoconstriction. High myoglobin level increasing F2-isoprostane quantity. Fluid sequestration and afterward increasing renal blood flow, activate RAAS. ROS production leads to endothelial dysfunction and then to an imbalance between vasoconstrictors and vasodilators substances. All these factors promote vessel spasm and its consequences [15, 18].

## DIAGNOSIS

Patients with RM may experience weakness, muscle and joint pain, fatigue, nausea with or without vomiting, diarrhea and many other complaints depends on the main cause of disease. In laboratory tests we can find elevated creatinine kinase (CK) levels – at least five to ten times beyond upper limit and electrolyte abnormalities such as hyperkalemia, hyperphosphatemia, hypocalcemia (at first), after cell destruction – hypercalcemia. Other blood chemistry findings are elevated myoglobin, transaminases, LDH. Serum CK levels gradually increase during the first 12 h, with a peak of 3–5 days [4]. If the myoglobin level in serum exceeding 0,3 mg/L, we have myoglobinuria in urinalysis with tea-colored urine. There is also a tendency to acidic Ph and detectable proteins in the urine sample [5].

When we considering which of those are the best AKI predictors the most often described is CK level [6, 7].

Another predictor that has also been used is myoglobin serum. In their cohort study, Premru and coworkers [6] found that >15 mg/L of myoglobin in the blood was highly associated with the development of AKI, but because of inconclusive data in other studies, it is not the best early prognostic. In the McMahon and co-worker's study, they created score includes among others: age, gender, etiology, and initial levels of creatinine, calcium, phosphate, serum bicarbonate as a tool to estimate mortality and AKI [11]. Initial CK and myoglobin levels are not corresponding with mortality.

We are currently looking for new markers that can be early predictors. Presently the two of them are on top of the list – neutrophil gelatinase-associated lipocalin (NGAL) and KIM-1.

NGAL is the most widely investigated biomarker – its production quickly increases, as a consequence of toxic or ischemic injury. In several studies, there is evidence that NGAL level could be used as a sensitive predictor of renal injury also correlated with mortality [13-14]. KIM-1 is quite well known as a biomarker increasing in humans with AKI secondary to ischemia due to aggravating body surgeries e.g. cardiac surgery.

However, there is still a lack of data to apply these early biomarkers from studies to clinical practice.

## TREATMENT

There are two main groups of therapeutic strategies – classics, such as well known fluid resuscitation, urine alkalization, diuretics or renal replacement therapies, and new strategies – based on administration anti-oxidant and anti-inflammatory substances or inhibitors vasoconstriction.

### *CLASSIC STRATEGIES*

FLUID THERAPY

Intensive fluid resuscitation is very important in RM-AKI. It should be administrated as soon as possible and should be carried out with monitoring diuresis rate to avoid overloading the circulation or hypovolemia, which can be also harmful. There are a few imprecisions in available studies but in most of them, we have evidence on lower short-term mortality or lower incidence of AKI in RM after receiving early fluids [19-20]. Michelsen and co-workers, in their review studies, make some recommendations to choose crystalloids instead of colloids because of lower risk of mortality [19]. Against a low level of evidence, they evaluate this recommendation as weak.

DIURETICS

Diuretic administration is still controversial. When, due to fluid therapy, we overhydrate the patient, the necessity of using them is obvious, but in other situations, there is still too little data. The most often we considering loop diuretics and mannitol. Mannitol increases urinary flow and prevents myoglobin precipitation. In some studies, there is data for reducing oxidative stress. Unfortunately, there are mice studies- no randomized trials were conducted on humans. The use of the loop diuretics (furosemide and torasemide) have not to be confirmed as necessarily specific in RM unless there is an additional reason [19, 21].

ALKALINIZATION

From our observation in clinical practice, urine alkalinization by using intravenous natrium bicarbonate is a popular therapeutic method. As the reason for this procedure, we can take available animal studies into account, which show inhibition of myoglobin precipitation and afterward reducing cast formation [22]. However, there is still a limited number of human research or they have been checking alkaline agents in various combinations, so it is impossible to value their results.

RENAL REPLACEMENT THERAPY (RRT)

High myoglobin level in blood circulation have a crucial influence to further damage. Hence, the assumption that its removal during renal replacement therapy should be a key importance to improve prognosis. Unfortunately plasma exchange, intermittent hemodialysis and conventional hemodialysis has limited evidence of effectiveness. Continuous veno-venous hemofiltration or hemodiafiltration with the super high flux filters and high volumes of hemofiltration have no data at all.

### *NEW STRATEGIES*

IRON CHELATORS AND ANTIOXIDANTS

Considering the mechanisms of damage, antioxidant and iron chelators may be included in the treatment, such as:

- desferrioxamine,
- vitamin E,
- vitamin C,
- acetaminophen,
- NAC (N-acetylcysteine),
- flavonoids.

As in many other cases, these therapeutic options still have too little data for their effectiveness. A list of potential mechanisms of action and available studies is presented in the table 1.

ANTI-INFLAMMATORY TREATMENT

- *Liposome-encapsulated clodronate (LEC)* – specific depletor of macrophages, which administration reduces apoptosis and preserve renal function (animal studies) by diminution the number of infiltrating macrophages [33-34];
- *Suramine* – polysulfonated naphthyl urea used in treating *sleeping sickness* disease; murine studies show a healing accelerating effect after AKI – after administration of this drug mice showed decreased KIM-1 (kidney injury molecule-1) expression, decreased tubular apoptosis and inflammation process by reducing NF-kB ( nuclear factor kappa-light-chain-enhancer of activated B cells) [15,35];
- *Pentoxifylline* – is a competitive nonselective phosphodiesterase inhibitor[9] which raises intracellular cAMP,

**Table 1.** AO – antioxidants and IC – iron chelators in RM-AKI treatment, NAC – N-acetylcysteine, ROS – reactive oxygen species MCP-1 – monocyte chemoattractant protein-1

| Substance | Category | Description | Evidence |
|---|---|---|---|
| Desferrioxamine | **IC** | lipid peroxidation inhibitor – reducing myoglobin to its ferrous form; hydrophilic – requires a lipophilic form to reduce nephrotoxicity [23]<br>alternative: *deferiprone* – the oral form used for ferrous overload by repeated blood transfusions | animal studies, no data about human trials<br><br>no data available |
| Vitamin E | **AO** | the major lipophilic antioxidants present in cellular membranes and protecting them against lipid peroxidation – theoretically could prevent myoglobin tubular toxicity – impediment: liposolubility implies a low ability to prevent myoglobin oxidation in the urine | animal studies with some efficiency to decrease toxicity [24-26], no animal/ human clinical trials |
| Vitamin C | **AO** | water-soluble;<br>reduce oxidative stress and inflammation by (theoretically) blocking the oxidation of myoglobin in urine and antiinflammatory role by inhibiting MCP-1 production [27]<br><br>synergic effects: *polyphenols*<br>lipid-soluble | animal (murine) studies with some efficiency to decrease toxicity [24-26], no animal/ human clinical trials |
| Acetaminophen | **AO** | inhibits lipid peroxidation by reducing ferryl myoglobin and urinary level of F2-isoprostanes<br>some studies have shown benefits not only in prophylaxis but in treatment [28] | mostly murine or rat studies,<br>no clinical data for benefits in human |
| NAC | **AO** | preventing cellular apoptosis by decrease in urinary thiobarbituric acid reactive substances (TBARS) concentrations, a lipid peroxidation marker, and inducing extracellular-signal-regulated kinase (ERK)pathway [31] | lots of research available, but proven efficacy only in animal models [29-30] |
| Flavonoids | **AO** | Probably electron donors with B-ring conjugated chemical structures rich in hydroxyl groups, which have potent antioxidant actions by reacting with and inactivating superoxide anions, oxygen lipid peroxide radicals, and/or stabilizing free radicals [32] | animal studies – largely concentrated on the influence on nerve cells, little data on the effect in people suffering from kidney damage from RM |

activates protein kinase A, inhibits TNF alpha, and leukotriene production, and decreases inflammation. In rats inhibits mesangial cells proliferation and myofibroblasts differentiation [15, 36];

- *MSC (mesenchymal stem cells)* – studies suggested that administration of MSC promotes kidney accumulation of protective macrophages, increased IL-10 level (anti-inflammatory interleukin), and reduce pro-inflammatory Il-6 and TNF-alpha [37].

NEW EXTRACORPOREAL BLOOD FILTER, CYTOSORB®

Referring to the different elimination techniques during dialysis, the trials are still underway to find a new type of filter to remove harmful myoglobin from the bloodstream, and thus reduce the frequency and severity of RM-AKI. The Cytosorb® – high-polymer filter with 300ml volume, low-resistance, and molecule selection <55 kD – has promising results caused by adsorbing cytokines such as Il-6, Il-8, and TNF-alpha or myoglobin (17 kD) [38]. The first reports of efficacy in patients with rhabdomyolysis have already appeared, but currently, the most data has been collected on the degree of cytokine reduction in patients in septic shock treated with CRRT only vs Cytosorb [39].

As molecules such as IL-10 are also involved in the uptake of the Cytosorb®, the overall immunomodulatory effect requires further research [40].

## CONCLUSIONS

Mechanisms that lead to kidney damage in rhabdomyolysis are complex and must be considered in a multidirectional manner. It seems that the main harmful factor is kidney hypoperfusion secondary to fluid sequestration and vasoconstriction. Not without significance is the effect of increased myoglobin level, leading to intratubular precipitation and activation of the inflammation cascade through the influence of its decay products and afterward increasing the pro-inflammatory agents. The ideal would be to predict and prevent rhabdomyolysis at all, but when it is impossible, the key to successful treatment is its rapid implementation. Therapy should be selected individually, adapting to the triggers, and closely monitoring the patient's condition. Early implementation of fluid therapy appears to be crucial. In addition, electrolyte

disturbances should always be detected in the early stages and carefully treated. The use of bicarbonates or diuretics may also be helpful, but especially in the latter case, the indications should be well evaluated, remembering to avoid hypovolemia. RRTs are most often implemented due to water-electrolyte or acid-base disorders. Due to myoglobin kinetics, not all renal replacement methods will be effective. They seem to be the greatest hope of high cut-off treatments, but these are still unproven and require further investigations. Similarly, new diagnostic methods and strategies based on the molecular mechanisms of inflammation required long-term, adequately designed randomized studies.

Until that, classical supportive therapeutic should be recommended.

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REVIEW ARTICLE

# FEATURES OF CRIMINAL LIABILITY OF A MEDICAL PROFESSIONAL FOR FAILURE TO PERFORM OR IMPROPER PERFORMANCE OF THEIR PROFESSIONAL DUTIES IN UKRAINE

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**ABSTRACT**

**The aim**: Research of features of criminal responsibility of the medical worker for failure to performe or imptoter perfomance of their professional duties in Ukraine.

**Materials and methods:** The article uses general scientific and special scientific methods of cognition, which provided an objective analysis of the research goal.

**Conclusions**: Criminal law, which provides for liability for improper performance of duties by a healthcare professional or pharmacist, must have a perfect design to ensure the rights and interests of both the patient and the medical worker.That is why, the existing construction of article 140 of the Criminal code of Ukraine requires a number of changes and additions.

**KEY WORDS:** medical worker, professional responsibilities, patient, minor, serious consequences

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## INTRODUCTION

Now the society has passed the centuries-old development is at the stage of the information society. Many standards, norms and principles relating to human rights and their significance have now been developed and implemented. Today the problem of ensuring human rights is not just national, but international. Many international, governmental and public organizations have been created in the world, whose activities are aimed at ensuring and implementing human and civil rights and freedoms. The universal Declaration of human rights is the main document that enshrines the right to life and medical care that is necessary to maintain her health [1].

In democratic countries, human life and health are recognized as the highest social value [2, p. 2402]. Ukraine, as part of the international community, enshrines and details these provisions in its domestic legislation. Thus, according to the Basic Law, Ukraine is a legal state where a person, his life and health, honor and dignity, inviolability and security are recognized as the highest value,. According to this list, human life and health are the main objects for protection in the state. This provision is specified in many articles of the Constitution of Ukraine. In particular, article 27 enshrines the right to life, and article 49 of the Constitution establishes the right to health protection and medical care. The state creates conditions for effective and accessible medical care for all citizens [3].

The priority nature of these constitutional provisions is enshrined in many legal acts. The key of effective guarantee, implementation and expression of priority value is the criminal legal protection of certain public relations. Current practice proves the fact that there is obvious complex

interconnection between medicine and law. Having long-lasting history of their establishment along with the contemporary high-profile sensitive cases, as well as the significant amount of various scientific researches clearly reflects that the issue of their correlation certainly remains a focus of public attention, especially when it comes to medical professionals, their actions and liability [4, p. 2573]. Moreover, the structure of the special part of the Criminal code of Ukraine is developed in accordance with the role of objects of protection for society. The second section of the Special part of the criminal law of Ukraine "Crimes against life and health". All crimes in this section are aimed to punish those who infringe on the basic natural rights of a person. A number of them establish criminal liability for medical workers as special subjects whose activities are specifically aimed at maintaining or restoring human life. The current criminal law contains a number of articles the subjects of which are medical workers, in particular, improper performance of professional duties by a medical worker, which resulted in infection of a person with the human immunodeficiency virus or other incurable infectious disease (article 131 of the Criminal code of Ukraine); disclosure of employee medical information, medical examinations to identify infection with the human immunodeficiency virus or other incurable infectious diseases (article 132 of the Criminal code); unlawful abortion or sterilization (article 134 of the Criminal code of Ukraine); failure to assist a patient health worker (article 139 of the Criminal code); improper execution of

professional duties by medical or pharmaceutical workers (article 140 of the Criminal code); violation of the rights of the patient (article 141 of the Criminal code of Ukraine); illegal conduct of experiments on a person (article 142 of the criminal code); forced donation (article 144 of the Criminal code of Ukraine); illegal disclosure of medical secrets (article 145 of the Criminal code of Ukraine). We would like to focus special attention on the study of the features of bringing a medical professional to responsibility for improper performance of professional duties and distinguishing this criminal act from related ones. After all, the construction of this legal norm must meet a number of requirements. At first to protect patients from arbitrariness or inaction of medical workers. At second to protect medical workers themselves from unfounded accusations.

## ANALYSIS OF RECENT RESEARCH AND PUBLICATIONS

Many autors reserched features of criminal responsibility of the medical worker for failure to performe or imptoter perfomance of their professional duties. For example, L. Schwartz, O. Paramonova, I. Fil, L. Karpenko, J. Marcelin and many others. However, there is no comprehensive scientific study of the analyzed crime, which would take into account all changes in the legislation.

## THE AIM

The aim of the article is providing characteristics of criminal liability of the health care professional for failure to performe or imptoter perfomance of their professional duties.

## MATERIALS AND METHODS

In this article, the authors used both general scientific and specially scientific methods. In view of the topic, purpose and objectives of the study, a dialectical method was used to interpret the conceptual and categorical apparatus of the research; the method of scientific analysis and synthesis has been used to investigate the constituent elements and features of the composition of the crime failure to performe or imptoter perfomance of their professional duties by medical worker; the formal and legal method is used to analyze the current legislation on liability for the analyzed crime. The authors also used a system-structural method that allowed them to analyze all the concepts systematically (in relation to and interconnected with other legal concepts). These and other research methods were used in this research in interconnectedness and interdependence, which ensured the comprehensiveness and completeness of the research, the truth of the obtained scientific results.

## REVIEW AND DISCUSSION

Analyzing official statistics, it was found that the most common crime that is committed by medical workers is an improper performance of professional duties by a medical or pharmaceutical worker. So, in 2018, 99 criminal proceedings were registered, and in 2019 – 130 [5]. Moreover, we are convinced that given the high latency of all crimes committed by special subjects and the level of their concealment by other colleagues, the official statistics are significantly different from the real ones. The upward trend is driven by many factors, both subjective and objective [6, p. 877]. After all, there is no single concept of medical error; medical personnel are prosecuted only if they made an unjustified error in the presence of all mandatory elements of the crime under the relevant article of the Criminal Code of Ukraine. At the same time, it is extremely difficult to prove the existence of such an error. In addition, at the state level, the causes and mechanisms of errors are not identified, they are not even discussed, which makes it impossible to outline measures to prevent them or reduce the frequency and degree of danger. In addition, there is no clear legislation in Ukraine that would explain what should be considered improper performance of professional duties, the rights and obligations of specific medical workers are very blurred in places, and the necessary equipment and drugs to provide qualified care are often not available on the ground. Moreover, the level of material support for doctors remains unsatisfactory. At the same time, there is another problem: law enforcement agencies are required to register all reports of a crime within 24 hours of their receipt. However, both often patients themselves and their relatives because of the image, misperception of the situation, or due to a biased attitude towards a particular doctor or the profession of doctor in general don't interprete his methods of diagnosis and treatment properly. This leads to the unjustified statements and messages on Commission of crime, which later areclosed for the lack of evidence [7, p. 39].

According to article 2 of the Criminal code of Ukraine, the only basis for criminal liability of a person is the commission of a socially dangerous act that contains the elements of a crime provided for in this Code. Unfortunately, the level of legal culture and legal awareness in Ukraine, both among medical professionals and the entire population, is at a very low level. That is why the vast majority of medical professionals have only a superficial idea of the legal liability that is established by current legislation for violations in the field of health protection. At the same time, knowledge of the grounds, types and consequences of legal liability, on the one hand, disciplines medical professionals, and on the other – reduces the likelihood of unjustified prosecution of them [8].

It is advisable to analyze the composition of each crime through the prism of its objective and subjective features. And the first of them is the object of the crime, that is, those social relations that were harmed or threatened by the crime. The analyzed crime has two objects of encroachment. The first of them (the main one) is human life (the right to life is a fundamental, inalienable, absolute human right, which means that the state guarantees protection from attacks on life and deprivation of life, maintenance of life and promotion of its extension) [9, p. 20] and human

health (a state of complete physical, mental and social well-being, and not only the absence of diseases and physical disabilities) [10].

The additional object of the crime is the established procedure for performing medical and pharmaceutical workers ' professional duties. To clarify the essence of the concept of "procedure for medical and pharmaceutical workers to perform their professional duties", it is important to note that Ukraine is currently in a state of significant reforms in the medical industry, when new approaches to the capacity of medical workers and the boundaries of their delinquency are just emerging and beginning to develop. That is why it is now important to introduce an effective mechanism that could protect both the rights of patients and clearly and objectively outline the cases of responsibility of medical professionals. After all, the existing wording gives grounds to regard any violations as criminal. In addition, O. Paramonova believes that the essence of this crime is the wrong illegal performance of medical and pharmaceutical workers of their professional duties. Therefore, the direct object of the act can be defined as public relations that ensure the right of citizens to qualified medical care [11, p. 246].

The victim is also singled outin the structure of the object of crime.The victim is a patient in this crime. A significant drawback of this article is the use of the very concept of "sick person" while most legal acts, given that medical care is also considered as a type of public service, use the term "patient". Such terminological differences are absolutely unacceptable in the field of criminal, the strictest legal liability, and in practice can lead to different law enforcement, which negates both the main international and domestic legal bases. We are convinced that the unification of concepts is a priority for the legislator. We consider it is necessary to use the term "patient" – an individual who has applied for medical assistance and / or who is receiving such assistance [10].

In addition, the victim of a crime under part 2 of article 140 of the Criminal code of Ukraine is a minor. Despite the fact that according to the Civil code of Ukraine, a minor is a person between the ages of 14 and 18, in this article it is still necessary to use not a literal, but an extended interpretation, and the victim may be a person under 18 years of age.On the objective side, the crime provided for in article 140 of the Criminal code of Ukraine is characterized by two acts, socially significant consequences and a causal relationship between the acts and the consequences. The first act is a failure to perform professional duties. By and large, non-compliance predictsthat a person does not perform the duties assigned to him, although he should have and could. The second action is improper execution, that is, although the person performs certain actions, but implements them superficially, not in full [12, p. 85].

Since the disposition of the article under investigation has a blank character, it should be established which professional duties were assigned to the medical worker and which of these duties were not performed at all or were not performed properlyin each specific case, as well as the requirements of which specific regulations (instructions, rules, instructions)were initiated by the suspect. It should be borne in mind that the diagnosis and the choice of a particular method and method of treatment depend on many factors. In particular, the individual characteristics of the patient's body, the achievements of medical science and experience of therapeutic activity. Therefore, they cannot be fully regulated in advance by health regulations [13, p. 70].

Regarding the consequences of the investigated action, the legislator clearly indicates that they should be severe. At the same time, the concept of "grave consequences" is estimated and will be determined by the court independently in each specific case. Thus, from the analysis of judicial practice, it follows that they traditionally recognize several types of consequences. First, bodily injury is a violation of the anatomical integrity of tissues, organs and their functions that occurs as a result of the action of one or more external damaging factors –physical, chemical, biological, and mental. According to the rules of forensic medical determination of the severity of injuries in the case of improper medical care, which resulted in a violation of the anatomical integrity of tissues and organs and their functions, the expert Commission has the right to consider this violation as a bodily injury and determine its severity By the rules of forensic medical determination of the severity of injuries [14, p. 78].

In Ukraine, injuries are: mild, moderate and severe. If we analyze the sanctions of the articles that stipulate the general conditions of liability for each of these types of injuries, they are more severe. In our view, this legislative approach is quite balanced, since; first and foremost, health care providers are a specific category that the state is obliged to provide qualified health care. At the same time, any person in any sphere of life is not protected from mistakes. Secondly, the fault of the medical worker before the act was committed is not intentional, but especially with regard to the consequences.

Secondly, the consequences of committing a crime under article 140 of the Criminal code of Ukraine may be the death of the victim caused by inaction or careless actions of a medical professional. Third, bringing the victim to suicide. For example, the perpetrator (the doctor) informed the patient that he was suffering from an incurable disease and, without wishing to do so, caused a mental health disorder, which led to suicide. The peculiarity of this feature of the crime is that it contains two socially dangerous consequences: the primary-a mental health disorder of the victim and the derivative-death. Suicide in the General psychological aspect is understood as human behavior aimed at self-destruction. The result of suicide is the biological death of a person. The subject of the crime treats both consequences carelessly [11, p. 188].

In the context of the amendment of the text of the disposition of the article of Art. 140 of the Criminal Code of Ukraine it seems appropriate to replace the phrase «severe consequences» with «severe consequences for the patient's health», which will help to better understand the meaning

of negative consequences that lead to legal liability for improper performance of professional duties by a medical or pharmaceutical worker, and will help to exclude from the content of this article all those consequences which do not concern life and health of the patient [15, p. 10].

Since the performance of professional duties is in most cases a certain process, it is important to establish the time of the end of this crime. The act provided for in article 140 of the Criminal code of Ukraine is formulated in the law as a crime with material composition. With regard to this issue, this means that one or another form of therapeutic effect acquires all the features of the objective side of the act provided for in article 140 of the Criminal code of Ukraine, only from the moment when the failure or improper performance of professional duties by a medical or pharmaceutical employee is manifested in a negative result, described in the law by the term " grave consequences for the patient [16, p. 297].

The subject of the crime is special, that is, a medical or pharmaceutical employee. The definition of the term "medical worker" is contained in the Ethical code of a doctor of Ukraine. At the same time it is completely wrong to identify a medical professional with a doctor [17, p. 199].

We are convinced that such a subject can be personswho have special education and meet the Uniform qualification requirements established by the Ministry of health for persons engaged in certain types of medical and pharmaceutical activities, including in the field of folk and alternative medicine [16, p. 299]. It should also be noted that the qualification is not affected by the form of the ownership to which belongs the hospital, where non-medical care is provided: public, municipal or private property. For a person to be recognized as the subject of this crime, he or she must meet not only General criteria: an individual, sanity, reaching a certain age, but also meet a number of special ones. The first of them is the availability of special medical education. The procedure for obtaining medical education, especially with regard to higher medical education, differs significantly from the procedure for obtaining education in other areas and specialties. The requirements and level of responsibility for this profession give rise to the increased selection criteria for training (increased level of results of external independent evaluation in specialized subjects); increased duration of training; training in an indivisible cycle, and so on. Thus, training, retraining and professional development of medical and pharmaceutical workers are carried out by the relevant secondary special and higher educational and scientific institutions, institutions of professional development and retraining, as well as through internships, medical residency, clinical residency, postgraduate and doctoral studies in accordance with the legislation on education [8]. That is, the qualification of the crime is not affected by what kind of secondary or higher education the medical worker has. That is, the subject of this crime can be a doctor, regardless of his profile, a nurse, or a paramedic.

We believe that when training young specialists, special attention should be paid to the formation of an unbiased and objective attitude to all patients, regardless of skin color, race, religion, sex or property qualification. Because now, numerous data about the unconscious influence of personal preferences on the process of providing care, interaction with the patient, etc. [18, p. 70]. This leads to the conclusion that a doctor can apply risky therapies in order to save a patient's life, keep an organ in function, etc., by first comparing the risky action and its potential outcome [19, p. 2411].

The second feature is compliance with the unified qualification requirements. Thus, a significant achievement is the existence of a unified State standard of higher medical and pharmaceutical education, which is the key to a unified, standardized approach to training qualified medical and pharmaceutical workers. Moreover, despite the fact that this standard is approved by the Ministry of Education and Science, but with the assistance and coordination of the Ministry of Health. This fact reaffirms that the standard meets the practical requirements of the profession and provides not only the preparation of general theoretical competences, but also specific practical skills.

The third sign is the presence of a special permit or license certifying the right to practice medical activities. A permit is required for those medical or pharmaceutical workers who have received education abroad, but a license is required in the case of medical activities by individuals who are business entities [20, p. 327].

In addition, the subject of this crime is persons engaged in alternative medicine, that is, healers (individuals-entrepreneurs who are engaged in folk medicine (healing) and received a special permit to practice folk medicine (healing) in accordance with the law to provide medical care to patients using traditional medicine methods based on the experience of many generations of people, established in folk traditions and do not require state registration) [21].

The last element of the composition of the investigated crime is its subjective side, which is characterized by a careless form of guilt, both criminal negligence and criminal self-confidence. In particular, carelessness can be expressed here in the form of criminal arrogance, in which a person foresees the possibility of the occurrence of socially dangerous consequences of their activities, but frivolously expects to prevent them. Frivolous calculation is usually based on the presence of certain professional abilities, skills, and experience in treatment. In the case of criminal arrogance, the determination of a person to perform actions to achieve the goal is connected with the calculation to prevent the occurrence of socially dangerous consequences. The guilty person may also fail to foresee the occurrence of socially dangerous consequences of their activities, mistakenly believing that they are unable to cause a socially dangerous result. In this case, the careless form of guilt manifests itself in the form of criminal negligence [16, p. 298].

In the context of the correct qualification of the acts of the person it is important to take into account the delimitation and abcence of the assistance to the patient by the medical officer, (article 139 of the Criminal code of Ukraine) medical or pharmaceutical workers (article 140

of the Criminal code of Ukraine). In our opinion, these articles are inconsistent with each other, because they are extremely similar. The only sign that allows them to differentiate is the form of guilt, so for article 139 of the Criminal code of Ukraine it is intentional, and for article 140 of the Criminal code of Ukraine-careless. For this reason, the legislator should consider the expediency of the existence of two norms and the possibility of introducing one of the remoteness of deliberate and careless guilt in two parts of the same article.

With regard to the punishment for committing a crime under Article 140 of the Criminal Code of Ukraine, we analyzed 65 court decisions included in the Unified State Register of Court Decisions of Ukraine for the period from 2011 to June 2020, according to which 67 medical workers were charged with crimes, under Art. 140 of the Criminal Code of Ukraine. Of these health workers, the majority (36 doctors and 3 nurses) were found not guilty and acquitted, and only 26 were found guilty by a court. However, only 10 persons were sentenced to actual punishment: 3 to imprisonment, 4 to restriction of liberty, and 3 to a fine. An additional penalty of deprivation of the right to engage in medical activities was imposed on 8 persons. Out of 10 convicts, only 2 were sentenced to more than 2 years in prison. Instead, most people were simply released from prison [22]. For example, one of the sentences on the accusation of a doctor under Part 1 of Art. 140 of the Criminal Code of Ukraine was acquittal (case /2018 / 1-47 / 11). The court ruled that the pre-trial investigation authorities had not proved, and the court session had not established, that during the victim's treatment there were circumstances that gave grounds to believe that the defendant had a thrombosis disorder, which subsequently led to her death. .Thus, the defendants met the requirements for the treatment of the patient, with the exception of certain laboratory tests, the absence of which could not affect the course of injury [23]. Instead, in another case (case № 106/11825/2012), the defendant, after conducting an initial examination of the victim, underestimated the severity of the traumatic factor (falling from a height of 4 floors), the severity of injuries and the severity of the general condition of the victim. Defects found in the victim's ribs and pelvic bones, the defendant mistakenly regarded as "outdated". Neurological disorders are mistaken for manifestations of cerebral atherosclerosis. In order to clarify the diagnosis, the doctor did not give instructions to medical staff for emergency catheterization of the bladder, electrocardiogram, not called for consultation neurosurgeon (neurologist). Underestimating the severity of the patient's condition, the doctor did not take the necessary measures to treat the patient, namely: immediate transfer of the victim to the shock room or intensive care unit with immediate initiation of infusion-transfusion therapy and other anti-shock measures. It should also be noted as a wrong referral of the patient for radiography, due to the fact that additional transportation and transfer could aggravate the patient's condition. These actions were qualified by the court under Article 140 of the Criminal Code of Ukraine – non-performance or improper performance by a medical or pharmaceutical worker of their professional duties due to negligent or dishonest treatment [24]. Thus, the judicial practice of Ukraine follows the path of imposing a medical negligence penalty in the form of imprisonment only in extreme, exceptional cases, mainly limiting it to milder measures of influence or even freeing such persons from serving their sentences [26, p. 2164].

Analyzing the judgments against Ukraine rendered by the European Court of Human Rights, it can be noted that the court continues to record the constant non-compliance of the Ukrainian authorities with patients' rights. In the decisions of the European Court of Human Rights in the cases «Kushnir v. Ukraine» (2014), «Temchenko v. Ukraine» (2015), «Savinov v. Ukraine» (2015), «Serhiy Antonov v. Ukraine» (2015 ), «Yaroshovets and others v. Ukraine» (2015), «Korneykova and Korneykov v. Ukraine» (2016), «Barsukov v. Russia» (2017) and others, improper performance of professional duties by medical or a pharmaceutical worker is recognized as inhuman and degrading treatment within the meaning of Art. 3 of the Convention for the Protection of Human Rights and Fundamental Freedoms [26].

In particular, in case Barilo v. Ukraine (application no. 9607/06) the applicant, who was suspected of having committed an official crime, had the status of a disabled person of group 3, suffered from diabetes mellitus and others chronic diseases. The applicant was in constant need of insulin injections, a special diet and constant medical supervision, which could not be provided in places of detention. Despite these arguments, the applicant was placed by the authorities at the Saki ITT for 10 days. According to the applicant, between 12 and 16 February 2006 she had injected herself with insulin because the ITT paramedic was on leave and the ambulance refused to come to the ITT. Immediately after her release, the applicant was hospitalized at the Evpatoria City Hospital, where she was diagnosed with diabetes mellitus, severe and suspected of having diabetic precoma (a condition preceding diabetic coma). Her health has deteriorated significantly.The court found that the applicant had not been provided with adequate medical care during her detention and that the conditions of her detention at the ITK in Saki constituted inhuman and degrading treatment. Although the applicant had been in the above conditions for only 10 days, she had suffered, which had greatly worsened her already poor health [27].

Improper performance of professional duties by a medical or pharmaceutical worker can be carried out at the general social, special criminological and individual levels. The purpose of these measures is to influence the causes and conditions of improper performance of professional duties by a medical or pharmaceutical worker, as well as to reduce the commission of this crime in the future [28, p. 17].

## CONCLUSIONS

Finally, we believe that criminal law, which provides for liability for improper performance of duties by a medical or pharmaceutical worker, should have an ideal design that

ensures the rights and interests of both the patient and the healthcare professional. Therefore, the existing structure of Article 140 of the Criminal Code of Ukraine requires a number of changes and additions. In particular, replace the concept of "sick person" with the concept of "patient"; do not equate a medical worker exclusively with a doctor.

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CASE STUDY

# A CASE OF MRI-NEGATIVE HERPES VIRUS ENCEPHALITIS PRESENTED BY RAMSAY HUNT SYNDROME

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**ABSTRACT**
Ramsay Hunt syndrome (RHS) occurs due to reactivation of latent Varicella Zoster Virus (VZV) infection in the geniculate ganglion of the facial nerve. Major clinical symptoms include ipsilateral facial paralysis, otic pain, and herpetic vesicles (rashes) along the nerve with accompanying ear pain. Rarely clinical findings include retrograde transaxonal spread of the virus from the ganglion into the brain parenchyma with developing the encephalitis or multiple cranial nerve involvement. We describe here a patient with both RHS along with complicating brainstem encephalitis developed due to the coinfection of VZV and EBV.

**KEY WORDS:** Ramsay Hunt syndrome, encephalitis, VZV, facial paralysis

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## INTRODUCTION

The rare clinical picture known as Ramsay Hunt syndrome was first described in 1907 [1]. The reason for the syndrome is a reactivation of varicella zoster virus (VZV) in sensory root ganglia. The geniculate ganglion is located on the facial nerve in the depths of the internal auditory meatus at the entrance to the bony fallopian canal. The infection involves facial and vestibulocochlear nerves, causing peripheral facial paralysis, otalgia and sensorineural hearing loss. Vestibular and meningeal symptoms, as well as brain parenchyma involvement are rare [2,3]. The most common complications of the disease are the facial paralysis and sensorineural hearing loss developed due to the involvement of the 8$^{th}$ cranial nerve. The general clinical symptoms as nausea, vomiting, vertigo are often present. In the literature, the number of cases with RHS complicated with multiple nerve involvement, and encephalitis is very rare [4-6].

## CASE REPORT

A 56-year-old female patient sought our emergency service of the Center of Infectious Disorders (CID) with the clinical complaints of inability to close the right eye, tears, and right-sided hearing loss associated with general weakness, malaise, labial deviation to the left. The symptoms had begun one month before her admission when there were pains in the right ear accompanied by rashes on the auricle. Soon right facial paresis joined. She went to a regional clinic where she was diagnosed with Ramsay Hunt syndrome (RHS, herpes zoster oticus) and treated accordingly. Since the condition did not improve, she turned to our Center, where the IgM VZV antibodies were found, after which she was hospitalized.

On admission, the general condition of moderate severity. Active, contact, adequate. Normal skin and mucous color. No abnormalities were found in the general examination.

Neurological exam indicated right cranial nerve VI and VII palsies House-Brackman grade IV, conductive hearing loss on the same side, skew deviation, and herpetic rashes and crusted lesions on the right auricle. There is a pain in the points of exit of the facial nerve branches on the right, violation of the sense of touch in the right half of the face. There was no nystagmus. Tendon and periosteal reflexes alive D≥S on the hands, and D≤S on the feet. There was a tremor in her hands during a complicated Barre-probe. Stryumpel, Sharapov-Raskolnikov, Chaddock, Pussep, and Barre symptoms were negative. Babinsky symptom was positive on both sides. Meningeal symptoms were not detected. She performed the coordination tests with intent, staggering in the Romberg pose. General blood test showed no significant changes. Clinical findings showing that RHS in our patient may also involve brain tissue forcing us to expand the diagnostic workup. A lumbar puncture was performed just on admission and PCR analysis revealed in CSF 421 734 copies per ml of EBV DNA. Magnetic resonance images of the brain performed at different modes (T1, T2, FLAIR) showed the unremarkable study.

Final diagnosis was right-sided Ramsay Hunt syndrome with liquor-dynamic disorders, vestibulo-ataxic, and psychostenic syndromes, associated with herpesvirus infection (VZV, and EBV in phase of activation), protracted course of moderate severity.

After diagnosis, our patient received CYMEVENE and MEDOVIR under the protocol, and dexamethasone ac-

companied by a variety of steroid-sparing agents. Control CSF analysis for EBV DNA made after three weeks of treatment was negative. The patients' condition improved greatly and she was discharged home for further out-clinic care and rehabilitation.

## DISCUSSION

Unique features of our case consists in simultaneous reactivation of Varicella Zoster Virus and Epstein Barr Virus (EBV).

Diagnosis in the cases of concomitant RHS and encephalitis generally consists of the clinical features, brain MRI, and CSF analysis, all of which were used in this case to establish a diagnosis. However, the sensitivity of these tests has not been well established in the literature. In our case, all three diagnostic modalities were necessary due to the unusual nature of the presentation. Our patient presented with signs indicative brain parenchyma involvement considering the multiple cranial nerve signs. IgM VZV antibodies determined in blood indicates the activation of this virus. The most peculiar point of our case in comparison with most cases of RHS was that in our case, we observed an activation of another herpesvirus, Epstein Barr virus, with a huge amount of viral DNA copies in the CSF. MRI at any modes showed no changes in the brain. Though imaging was negative, clinical signs and CSF studies confirmed CNS involvement in our patient. Since, CNS involvement is unusual in Ramsay Hunt syndrome [2], and taking into account a high level of EBV DNA in liquor, we suggest that Epstein Barr virus has made the greatest contribution to the development of encephalitis.

## CONCLUSIONS

The treatment of RHS is the combination of antiviral drugs to stop virus replication and anti-inflammatory medicines. Early clinical suspicion was beneficial in starting aggressive treatment.

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