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PECULIARITIES OF THE PERIODONTAL POCKETS MICROBIOTA DURING ACUTE DURATION OF GENERALIZED PERIODONTITIS

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Periodontal inflammatory and inflammatory-dystrophic diseases are actual problems in modern dentistry, and the role of oral microbiota in periodontopathies` development remains unclear. The microbiota of periodontal pockets contents from 20 patients was studied (10 – by the polymerase chain reaction and 10 – by the bacteriological method). 6 out of 10 patients were diagnosed with periodontal pathogens in clinically significant concentrations. In 4 cases bacterial complexes of five representatives were determined with 2 main combinations – P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola and C. albicans, and P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola, and A. actinomycetemcomitans. There were no cases of simultaneous detection of C. albicans and A. actinomycetemcomitans. In 7 out of 10 cases periodontal pockets contained purulent aerobic and facultative anaerobic microorganisms in clinically significant concentrations.

Key words: dentistry, periodontium, inflammation, microorganisms, polymerase chain reaction, bacteriology.

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ОСОБЛИВОСТІ МІКРОБІОТИ ПАРОДОНТАЛЬНИХ КИШЕНЬ ПРИ ГОСТРОМУ ПЕРЕБІГУ ГЕНЕРАЛІЗОВАНОГО ПАРОДОНТИТУ

Запальні та запально-дистрофічні захворювання пародонту залишаються актуальними проблемами для сучасної стоматології. Ролі мікроорганізмів порожнини рота в розвитку пародонтопатій залишаються нез'ясованими. Було проведено дослідження вмісту пародонтальних кишень у 20 пацієнтів (10 – виконано полімеразно-ланцюгові реакції та 10 – застосовано бактеріологічний метод). У 6 з 10 пацієнтів було виявлено пародонтопатогени в клінічно значимих концентраціях. В 4 випадках були встановлені бактеріальні комплекси у складі 5 мікроорганізмів в 2 основних комбінаціях – P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola з C. albicans та P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola з A. actinomycetemcomitans. Не було випадків одночасного виявлення C. albicans та A. actinomycetemcomitans. В 7 з 10 випадків в пародонтальних кишенях виявлялася гноєрідна аеробна та факультативно анаеробна мікрофлора в клінічно значимих концентраціях.

Ключові слова: стоматологія, пародонт, запалення, мікроорганізми, бактеріологія, полімеразно-ланцюгова реакція.

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Chronic inflammatory and inflammatory-dystrophic lesions of periodontal tissues are widespread diseases both in Ukraine and throughout the world. The most common periodontal diseases have complex and multi-stage etiology and pathogenesis with multicomponent pathological reactions that should be noted both at the local level and at the level of the whole organism. Among the etiological factors in the development of generalized periodontitis in humans the leading role is assigned to the infectious factor which includes colonization and reproduction in the periodontal sulcus and periodontal pockets of especial microorganisms with pronounced periodontopathogenic properties. In the structure and qualities of the abovementioned bacterial colonies it is necessary to highlight the ability to cause and maintain the course of inflammatory, destructive processes in periodontal tissues. According to WHO data, about 20 representatives of the microworld (with high adhesive, invasive and toxic properties, mainly the obligate anaerobes) are classified as periodontopathogenic microorganisms. They are: Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Tannerella forsythia, Actinobacillus actinomycetemcomitans, etc. A number of performed clinical and laboratory studies prove more the complex and systemic mechanisms of action of these microorganisms' pathogens on the patient's body as a whole – from an increase in the level of cardiovascular diseases and the risk of sudden death, to the incidence of squamous cell carcinoma of the digestive tract, etc. [2, 4, 5, 7, 10].

S. S. Sokransky after performing a series of studies of the periodontal pockets microbiota in periodontitis, distributed the detected groups of microorganisms into periodontal complexes – "yellow", "purple", "green", "orange", "red" complexes and 3 microorganisms of non-complex organization [14]. According to the proposed gradation, the "green" complex consisted of the following bacteria: Eikenella corrodens, Capnocytophaga gingivalis, Capnocytophaga sputigena, Capnocytophaga ochracea, Campylobacter concisus, Aggregatibacter actinomycetemcomitans serotype a; "yellow" complex – Streptococcus mitis, Streptococcus oralis, Streptococcus sanguis, Streptococcus gordonii, Streptococcus intermedius; "purple" complex: Veillonella parvula, Actinomyces odontolyticus. Actinomyces naeslundii

(Actinomyces viscosus) is a non-clustered microorganism that is isolated in a separate "blue" complex. The most aggressive in terms of its effect on periodontal tissues is the "red" complex consisting of Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola. Some authors also include in this complex Actinomyces viscosus, Selenomonas noxia, Aggregatibacter actinomycetemcomitans serotype b, which are not non-clustered microorganisms [5, 11, 13]. Less aggressive is the pathogenic "orange" complex consisting of Prevotella intermedia, Prevotella nigrescens, Peptostreptococcus micros, Campylobacter gracilis, Campylobacter rectus, Fusobacterium periodonticum, Fusobacterium nucleatum (subsp.: Nucleatum, vincentii, polymorphum), Streptococcus constellatus, Eubacterium nodatumae, Campylobacter showae. Aggregatibacter actinomycetemcomitans serotype b is a non-clustered periodontopathogenic microorganism with a high level of virulence and pathogenic effect on periodontal tissues [6, 12].

Due to relevance of the issues of introducing modern methods of treating periodontal diseases in the population and the principles of biological safety control in the field of health care, it was decided to perform this study [1, 8].

The purpose of the study was to research the composition of periodontal pockets in patients with acute generalized periodontitis to establish its regional peculiarities using polymerase chain reaction and bacteriological methods.

Materials and methods. The study group was formed from 20 patients (12 females and 8 male) who obtained a complex periodontal treatment at the private dental medical center "Perio-center" in Kyiv, Ukraine. The mean age of the patients was 33.00±4.19 years (M=34.00). The all patients come to the dental clinic due to the needs of medical care for the acute course of generalized periodontitis II–III severity. After obtaining informed consent from patients, before starting treatment, the contents of periodontal pockets from inflammation zone was collected. Patients were randomly divided into two equal subgroups. In the first group, the "Parodontoscreen" study was performed (by polymerase chain reaction (PCR) on nucleic acids of aggressive periodontopathogenic microorganisms from periodontopathogen complexes (A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola) and C. albicans. The contents of the pockets were taken with a sterile disposable probe and fixed in a special transport medium (Eppendorf tube). The laboratory stage of this part of the study was performed at Medical laboratory LTD (Kyiv, License for medical practice of the Ministry of Health Care of Ukraine AE No. 571609). In the second sub-group of patients biological material was taken from the periodontal pockets in zone of active inflammatory process using a sterile transport system (a tube with AMIES gel and an applicator for biological fluids The material was seeded on nutrient media: chromogenic agar ("bioMerieux", France), "Sabouraund Dextrose Agar" ("Himedia") for the cultivation of microscopic fungi of the genus Candida; for haemolytic bacteria of the genus Streptococcus and Neisseria – on blood agar, for Enterobacteriaceae – on Endo and Ploskirev media; bacteria of the genus Staphylococcus were cultivated on Mannitol Salt Agar ("Biolif-Italia"), bacteria of the genus *Enterococcus* were cultivated on "Bile esculin agar" ("Biolif-Italia"). The pure culture of microorganisms was determined with sector inoculation method by the Gold. The identification of microorganisms was carried out due to the morphological, tinctorial, cultural and biochemical properties of bacteria using "ENTERO-test", "STREPTO-test", "STAPHYLO-test" ("Erba Lachema", Czech Republic). All stages of bacteriological studies were performed at the bacteriological laboratory of the Department of Genetics, Plant Physiology and Microbiology, Biological Faculty, Uzhhorod National University.

Results of the study and their discussion. The results of the studies of periodontal pathogenic microorganisms by PCR showed that among patients with exacerbation of generalized periodontitis representatives of the "red" complex of periodontal pathogenic microorganisms were determined. This pattern was observed in 9 cases out of 10 patients. The total number of microorganisms in the material was 10^4–10^8 Lg GE/ml, which indicates that a sufficient amount of the test material was obtained for the PCR-reaction.

The most common periodontal pathogen in the patients` group was T. forsythensis (B. forsythus) which was found in 8 patients. T. denticola was the second most common bacteria in the patients` group, it was found in 7 out of 10 patients. 6 patients of 10 had P. gingivalis, and half of the patients (5) had P. intermedia. A. actinomycetemcomitans and C. albicans were detected much less frequently – in 2 samples. The average frequency of determining at least one periodontopathogenic microorganism from the "red" complex was 50.00±20.00 % (M=55.00 %). Only in one case nucleic acids of the abovemebtioned periodontal pathogens were not found, which may indicate the participation of the other microorganisms in the development of the acute inflammatory process, since the number of certain microorganisms in the test sample was 10^7 Lg GE/ml. Evaluation of the composition of complexes of periodontopathogenic microorganisms made it possible to determine that only one representative was found in 2 patients, in one case it was T. forsythensis (B. forsythus), in the other case, T. denticola. Two more patients had complexes

of the two most aggressive periodontopathogens – P. gingivalis in combination with T. forsythensis (B. forsythus) and T. denticola in combination with T. forsythensis (B. forsythus). Complexes of 3 aggressive periodontopathogenic microorganisms were found in 2 patients, in one case they were combinations of P. intermedia, T. forsythensis (B. forsythus) and T. denticola, in the other – P. gingivalis, T. forsythensis (B. forsythus) and T. denticola. In 4 patients, the complexes included five aggressive periodontopathogenic microorganisms – in 2 out of 10 patients the bacteria complexes included *P*. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola and C. albicans. And in 2 samples the bacterial complex consisted of P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola, and A. actinomycetemcomitans. It should be noted that microscopic fungi C. albicans have never been found in combination with A. actinomycetemcomitans. In half of the patients from the study subgroup there were complexes with the participation of three periodontopathogenic microorganisms – P. gingivalis, T. forsythensis (B. forsythus) and T. denticola, in various combinations; and in 7 cases a pair of T. forsythensis (B. forsythus) and T. denticola was present in the assosiations.

The number of certain microorganisms in the material, their clinical significance**.** An assessment of the number of obtained nucleic acid replicas in the material suggested that individual microorganisms both independently and in combination were determined in clinically insignificant quantities. Namely, the C. albicans fungi found in two cases were in amounts up to 4.50 Lg GE/ml (all cases), in one of six cases of detection of P. gingivalis (their number was also less and amounted to 5.00 Lg GE/ml); in two cases out of five identified P. intermedia was also found to be up to 4.50 Lg GE/ml; in four cases out of nine, T. forsythensis (B. forsythus) was detected (the number of microorganisms was 5.00 Lg GE/ml); in two cases out of eight, when T. denticola was detected, the number of replicas was up to 3.50 Lg GE/ml. Based on the analysis of the obtained data, it is possible to single out cases of determination of nucleic acids of aggressive periodontopathogenic microorganisms in clinically significant amounts (table 1). And there were 6 out of 10 such cases.

Table 1

Analysis of cases of detection of periodontopathogenic microorganisms in clinically significant amounts, Lg GE*/ml

Case	Microorganism						Total amount of micro-organisms
	A. actino-mycetem-comitans	P. gingi-valis	P. inter-media	T. forsyth-ensis (B. forsythus)	T. den-ticola	C. al-bicans	
1		10^6	10^5	10^6	10^5	$^{**}10^4$	10^7
2				$^{**}10^4$	10^4		10^7
3		10^7		10^6	10^6		10^8
4	10^4	10^6	10^4	10^5	10^4		10^6
5		10^6	10^5	10^6	10^6	$^{**}10^3$	10^7
6	10^4	10^7	10^5	10^6	10^6		10^7

Note: *GE – genomic equivalent; ** the number of microorganisms did not reach a clinically significant level

Fig. 1. The results of sowing the contents of the periodontal pockets of patient K. on differential diagnostic nutrient media *(S. pyogenes, K. pneumoniae, C. albicans* and *E. coli* were identified)

Bacteriological studies. In the second subgroup of patients the contents of periodontal pockets were taken and followed by cultivation on differential diagnostic nutrient media (for aerobic and facultative anaerobic microflora). As a result, the following representatives were identified: Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Citrobacter freundii, Enterobacter cloaceae, Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus viridans and Candida spp (fig. 1, 2).

According to the frequency of detection, microorganisms were distributed as follows. S. epidermidis (with hemolytic properties) was isolated most often in half of the examined (in the amount of 3.0×10^2 colony-forming unit (CFU)/ml, 9.3×10^4 CFU/ml, 1.2×10^7 CFU/ml, 2.0×10^3 CFU/ml and 4.2×10^2 CFU/ml) and E. coli (3.0×10^5 CFU/ml, 11.5×10^5 CFU/ml, 5.3×10^5 CFU/ml, 1.2×10^2 CFU/ml and 9.0×10^3 CFU/ml). S. pneumoniae (4.8×10^5 CFU/ml, 2.3×10^5 CFU/ml and 1.0×10^4 CFU/ml), Candida spp. (2.4×10^3 CFU/ml, 3.2×10^3 CFU/ml and 3.0×10^6 CFU/ml) and S. aureus (1.2×10^5 CFU/ml, 1.2×10^2 CFU/ml and 3×10^7 CFU/ml) were found in three samples.

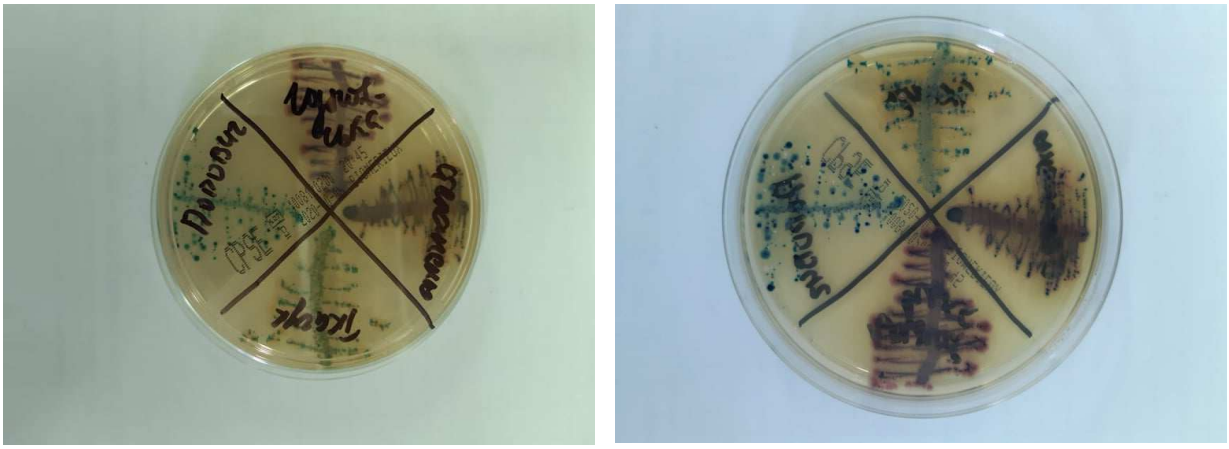

Fig. 2. Growth of the opportunistic microflora on chromogenic agar

Two variants per study group were identified for S. pyogenes (11.2×10^5 CFU/mL and 4.3×10^5 CFU/mL), S. viridans (5.3×10^5 CFU/mL and 2.5×10^5 CFU/ml), E. faecalis (1.4×10^2 CFU/ml and 2.8×10^3 CFU/ml), P. aeruginosa (2.0×10^8 CFU/ml and 3.0×10^5 CFU/ml) and K. pneumoniae (2.3×10^4 CFU/ml and 6.9×10^7 CFU/ml) and one case of detection of K. oxytoca (1.3×10^9 CFU/ml), E. cloaceae (5.0×10^5 CFU/ml /ml) and C. freundii (5.0×10^2 CFU/ml). Clinically significant numbers of microorganisms were found in 3 out of 10 patients for S. pneumoniae and E. coli, in 2 cases for S. aureus, S. epidermidis, S. viridans, P. aeruginosa, S. pyogenes and K. pneumoniae, and in one case for K. oxytoca, E. cloaceae, and Candida spp. (table 2).

Table 2

Frequency of selected microorganism detection (n=10)

Microorganism	Number of cases	Number of cases of microorganism detection in clinically significant concentration (10^5 CFU/ml and more)
S. aureus	3	2
S. epidermidis	5	2
S. pneumoniae	3	3
S. pyogenes	2	2
S. viridans	2	2
E. faecalis	2	0
P. aeruginosa	2	2
K. oxitoca	1	1
K. pneumoniae	2	2
E. coli	5	3
E. cloaceae	1	1
C. freundii	1	0
Candida spp.	3	1

Due to the absence of full information about mechanisms of periodontal diseases development in humans the studying etiological and multifactorial aspects of abovementioned problems is still relevant. Especial scientific interest may be presented into data about common role of complex presented by periodontopathogenic, relative pathogenic and saprophytic bacteria and fungi in development the periodontal tissues and alveolar bone lesions development [12]. Thus, a two-stage study of the microbial content of periodontal pockets in patients with acute duration of generalized periodontitis was performed in 2 stages – due the specifics of the cultural properties of periodontopathogenic microorganisms (aggressive periodontopathogenic strains of microorganisms are predominantly obligate anaerobes, which requires more complex and expensive methods of identification and cultivation), and the probability of inclusion of these representatives in the composition of associations of microorganisms and the formation of biofilms. In practice, the usage of PCR-identification methods improves the quality and accuracy of diagnosing periodontopathogenic microflora and minimizes the possible negative impact of the human factor and errors when performing such studies. Obtained results showed not total presence of periodontopathogens in subgroup during the acute inflammation. During the second stage, a number of classic bacteriological studies of the contents of periodontal pockets were carried out to identify aerobic and facultative anaerobic microorganisms using differential diagnostic nutrient media [3].

During of PCR-tests only individual representatives of the Sokransky`s periodontopathogen complexes and *Candida spp.* fungi were determined as the most aggressive microorganisms to periodontal tissues. Analysis of the results of the study showed the presence of representatives of the

periodontopathogenic complex in 6 out of 10 patients of the study group in clinically significant concentrations. Probably, the other not detected microorganisms caused the inflammation in periodontal tissues [1, 7]. In 4 cases out of 10, bacterial complexes of 5 representatives of periodontopathogenic microorganisms were determined, and these were two identical combinations each – the first option was P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola and C. albicans and the second variant – P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola and A. actinomycetemcomitans. There were no cases of simultaneous detection of C. albicans and A. actinomycetemcomitans what may be concerned as signs of natural antagonism. All new facts may by part for planning of further studies of multifactorial effects of different bacterial complexes on periodontal tissues lesions.

As a result of bacteriological studies of the contents of periodontal pockets in patients with acute duration of generalized periodontitis in laboratory the row of aerobic and facultative anaerobic pathogenic bacteria genus was detected, which may cause hard purulent inflammation separately and in associations – P. aeruginosa, S. aureus, S. pyogenes, S. epidermidis, E. coli, K. pneumoniae, K. oxytoca, C. freundii, E. cloaceae, E. faecalis, S. pneumoniae, S. viridans and Candida spp. But not high level of detection the clinically significant numbers of one genu of microorganisms during the inflammation may lead us to conclusions about not clear role of aerobic pathogenic and facultative anaerobic microflora in pathological process – as we determined only in 3 out of 10 cases for S. pneumoniae and *E. coli*, in 2 out of 10 cases for S. aureus, S. epidermidis, S. viridans, P. aeruginosa, S. pyogenes, K. pneumoniae, and 1 out of 10 cases for K. oxytoca, E. cloaceae and Candida spp. By the classic general pathology, multifactorial character of disease`s etiology may indicate about unknown causative factor. But anyway, the performed analysis of the obtained data of cultivation and identification of isolates of bacterial cultures of aerobic and facultative anaerobes showed that in 7 cases out of 10 during the acute duration of generalized periodontitis purulent microflora was present in periodontal pockets in clinically significant concentrations. The results obtained correlate with the results of studies of the microbiota of the inflammatory locus in generalized periodontitis published earlier in the specialized literature [9, 15].

Conclusion

Thus, the performed PCR-studies of the periodontal pockets contents made it possible to use laboratory methods to diagnose the presence of aggressive representatives of bacterial periodontopathogen complexes in 6 out of 10 patients with an acute duration of generalized periodontitis in clinically significant concentrations. In 4 out of 10 cases bacterial complexes of 5 representatives of periodontopathogenic microorganisms were determined, it should be noted the same combinations in two cases – the first option – P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola and C. albicans and the second variant is P. gingivalis, P. intermedia, T. forsythensis (B. forsythus), T. denticola, and A. actinomycetemcomitans. There were no cases of simultaneous detection of C. albicans and A. actinomycetemcomitans. Bacteriological studies using differential diagnostic nutrient media for the cultivation of aerobic and facultative anaerobic microorganisms showed that in 7 out of 10 cases with an acute duration of generalized periodontitis, pathogenic and opportunistic microbiota were present in periodontal pockets in clinically significant concentrations – in 3 out of 10 cases for S. pneumoniae and E. coli, in 2 out of 10 cases for S. aureus, S. epidermidis, S. viridans, P. aeruginosa, S. pyogenes, K. pneumoniae, and 1 out of 10 cases for K. oxytoca, E. cloaceae and Candida spp.

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LIPID PARAMETERS BEFORE AND AFTER IMMUNOBIOLOGICAL THERAPY OF PATIENTS WITH PSORIASIS

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Psoriasis is one of the most common dermatoses and occupies one of the leading places among the current problems of modern dermatology. 148 patients were involved in the study. Comparative evaluation of the therapeutic efficacy of monotherapy with the immunobiological drug Adalimumab, as well as its combination with a domestic non-hormonal drug based on natural ingredients (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) in the examined patients with psoriasis vulgaris was performed according to the dynamics of regression of cutaneous clinical manifestations of dermatosis: reduction of lesion area, regression of erythema, infiltration, peeling of psoriatic skin rash, changes in PASI, PGA, BSA index. We have proposed a modified treatment regimen for patients with psoriasis vulgaris, which provides a course of systemic immunobiological therapy with adalimumab with concomitant use of domestic non-hormonal drug can increase the effectiveness of treatment and prolong the remission of dermatosis..

Key words: psoriasis, features of lipid metabolism, immunopathogenesis, immunobiological therapy.

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ПОКАЗНИКИ ЛІПІДІВ ДО ТА ПІСЛЯ ПРОВЕДЕННЯ ІМУНОБІОЛОГІЧНОЇ ТЕРАПІЇ У ХВОРИХ НА ПСОРІАЗ

Псоріаз є одним з найбільш поширених дерматозів і посідає одне з провідних місць серед актуальних проблем сучасної дерматології. У дослідженя було залучено 148 пацієнтів. Порівняльна оцінка терапевтичної ефективності застосування монотерапії препаратом імунобіологічної дії Адалімумаб, а також його комбінації з відчизняним негормональним препаратом на основі натуральних компонентів (Лляна олія, солідол жировий, D-пантенол, алантоноїд, екстракт череди, екстракт чистотілу, екстракт оману, сірка, саліцілова кислота) у обстежених хворих на псоріаз вульгарний проводилась згідно динаміки регресу шкірних клінічних проявів дерматозу: зменшення площі ураження, регрес еритеми, інфільтрації, лущення елементів шкірної псоріатичної висипки, змін індексу PASI, PGA, BSA. Запропонована нами модифікована схема терапії хворих на псоріаз вульгарний, яка передбачає проведення курсу системної імунобіологічної терапії препаратом адалімумаб з паралельним призначенням відчизняним негормональним препарату дозволяє підвищити ефективність лікування та подовжити термін тривалості ремісії дерматозу.

Ключові слова: псоріаз, особливості ліпідного обміну, імунопатогенез, імунобіологічна терапія.

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The main characteristic of the pathological process is recognized as immune inflammation, accompanied by activation of T-lymphocytes and excessive production of mediators of the immune response. The pathological process is also characterized by an imbalance of lipid metabolism, in particular a decrease in the level of high-density lipoprotein (HDL) and an increase in low-density lipoprotein (LDL) [1]. The pathological process of cholesterol accumulation (cholesterol) triggers the production of pro-inflammatory