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Memory of  
dr Władysław  
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## ORIGINAL ARTICLE

## INTEGRATIVE MORPHOMETRIC CHARACTERISTIC OF ENDOTHELIAL DYSFUNCTION IN THE CASES OF CHILDREN WITH ESSENTIAL ARTERIAL HYPERTENSION

DOI: 10.36740/WLek202104125

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### ABSTRACT

**The aim:** To optimize the treatment of children with Essential Arterial Hypertension (EAH) in association with Endothelial Dysfunction (ED) by studying the clinical and morphofunctional characteristics of the cardiovascular system disorders and correction of endothelial dysfunction with the using of essential phospholipids.

**Materials and methods:** The study group consisted of 80 children and 30 – a control group. The next stage included the division of 80 children into 2 subgroups. Patients in the first subgroup received basic treatment (angiotensin-converting enzyme inhibitor of the third generation), the second – optimized treatment (basic treatment was with addition of certified drug lecithin). Doses were determined according to the instructions and age for 2 months. In the study were used: ECG, Echocardiography, Ultrasonography, Morphofunctional studies of the endothelium.

**Results:** There is a dynamic decreasing in the level of left ventricular myocardial mass index (LV MMI), reduction of end-diastolic volume (EDV) and increase in the absolute values of shock volume (SV), ejection fraction (EF) under the influence of optimized treatment due to the inclusion of lecithin in the treatment of children with EAH with ED. The Ve/Va ratio had a tendency to increase. Vasoconstriction of vessels after the reactive hyperemia test was significantly reduced, but the degree of vasodilation varied depending on the method of therapy. The intima-media thickness (IMT) decreased in 1.12 times in the cases of children with an optimized treatment, accompanied by a decreasing of DEC by 2-times. Levels of the aortic stiffness index had a tendency of decreasing (from  $0.88 \pm 0.02$  to  $0.71 \pm 0.01$  and to  $0.63 \pm 0.01$ , respectively, by groups and in comparison with the control group –  $0.55 \pm 0.01$ ), which reflects the improvement of hemodynamic parameters. The dynamic parameters obtained in the cases of patients with EAH in association with ED, taking into account the impact of the optimized treatment had positive correction on the total risk of cardiovascular complications, changes in the profile of LV diastolic filling, dysfunction of arterial endothelium.

**Conclusions:** The inclusion of essential phospholipids in the treatment of children with EAH and ED helps to optimize the profile of LV diastolic filling and exclude vascular endothelial dysfunction and indicate a positive effect of optimized treatment on the overall risk of cardiovascular complications.

**KEY WORDS:** Essential Arterial Hypertension, Endothelial Dysfunction, children

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### INTRODUCTION

Despite progress in the prevention, diagnosis, treatment and control of high blood pressure (BP), there is a further negative trend towards an increasing in the incidence of essential hypertension (EAH) in the pediatric population. Almost every third child with hypertension may develop hypertension disease in the future, which determines the importance of early diagnosis and prevention of hypertension, when the increase in blood pressure is not yet stable. However, clinical and pathogenetic features of EAH in childhood have not been studied enough. This is due to the presence of various pathogenetic mechanisms of EAH development and the peculiarities of its clinical manifestations in the cases of children and insufficient

study of the factors through which the main mechanisms of cardiovascular remodeling are realized [1,2].

The endothelium is an active endocrine organ, the largest in the body, diffusely scattered along with the vessels in all tissues. According to the classical definition of histologists, the endothelium is a single-layer layer of specialized cells that line the inside of the entire cardiovascular tree, weighing about 1.8 kg. This is one trillion cells with complex biochemical functions, including systems for the synthesis of proteins and low molecular weight substances, receptors, ion channels [3]. In a broad sense, ED is a condition of vascular endothelium that is accompanied by impaired vasotonic, remodeling, anti-inflammatory and anticoagulant functions. ED is the first link in the pathophysiology



of the cardiovascular continuum, which is based on progressive vascular damage, exacerbated by hypertension. Considering the functions of the endothelium, we can talk about a very complex mechanism of interaction of antagonistic mediators, as well as their regulation of various physiological effects. The spectrum of biologically active substances released by it, changes dramatically, when the function or structure of the endothelium is violated. Under adverse conditions, the endothelium becomes the initiator (or modulator) of many pathological processes in the body. Steady increasing in vascular tone leads to restructuring in the vascular wall [4]. Stages of structural changes are preceded by functional disorders caused by an imbalance in the ratio of vasoconstrictor and vasodilator reactions. Violation of this balance, which is manifested in the development of an imbalance between factors secreted by endothelial cells, is accompanied by the development of endothelial dysfunction.

### THE AIM

The aim to optimize the treatment of children with EAH in association with ED by studying the clinical and morphofunctional characteristics of the cardiovascular system disorders and the using of essential phospholipids for the correction of endothelial dysfunction.

### MATERIALS AND METHODS

The study group consisted of 110 children. At the first stage, 80 adolescents, aged 15–17 years (average age  $16.0 \pm 0.11$ ) were examined, among them were 32 boys ( $40.0 \pm 7.75$ ) and 48 girls ( $60.0 \pm 7, 75$ ), in cases of which endothelial dysfunction was identified. The average height of adolescents was  $172.0 \pm 0.99$  cm, which corresponds to 50 centiles, body weight –  $46.0 \pm 1.01$  kg, which corresponds to 10 centiles in children. 2 groups were formed: the first group consisted of 80 children with endothelial dysfunction, the control group included 30 healthy children with identical parameters.

The second stage included the distribution of the first group of children into 2 subgroups. Patients of the first subgroup used basic treatment (angiotensin-converting enzyme inhibitor of the third generation), the second – optimized treatment (basic treatment supplemented with a certified drug, lecithin). Doses were determined according to the instructions and age for 2 months.

ECG, echocardiography, ultrasonography, morphofunctional investigation of the endothelium were used in the study. The diastolic function of the left ventricle was investigated according to the method by L.K. Hattle, B. Angelsen, 1985 [5] in pulsed Doppler mode with mapping of the transmitral flow from the apical access of the heart. The assessment of LV diastolic function was performed on the indicators of flow velocities in the phases of peak early diastolic ( $V_e$ ) and peak late diastolic flow ( $V_a$ ), their ratio ( $V_e / V_a$ ). The aortic stiffness index was defined as the ratio between pulse BP (PBP) and stroke volume (SV).

For endothelial dysfunction identification, a test of reactive hyperemia was performed using a TOSHIBA Aplio 400 (device with a linear sensor with a frequency of 7.5 MHz). The children had to lie on their backs for at least 10 minutes, before the examination. Next, the longitudinal scan measured the diameter of the brachial artery (in the diastole phase) and blood flow velocity (average result for 4 cardiac cycles). Estimation of the diameter of the brachial artery 2 – 5 cm above the elbow flexion. An air cuff was placed on the upper third of the shoulder, creating a pressure that exceeded the systolic blood pressure by 50 mm. rt. art. (5 minutes). Next, the velocity of the indicators was calculated and the diameter of the artery was measured at 30 and 60 s after rheoocclusion. The criterion for the presence of endothelial dysfunction is an increase in the diameter of the brachial artery less than 10% [6].

The endothelial dysfunction was characterized by the following parameters:

V max – Maximum systolic (or peak) velocity is the actual maximum linear velocity of blood flow along the axis of the vessel, expressed in mm/s, cm/s or m/s.

V min – Minimum diastolic linear blood flow velocity along the vessel.

V mean – The velocity integral under the curve enveloping the blood flow spectrum in the vessel.

RI (Resistivity Index, index Purcell) – vascular resistance index.  $RI = (V_{\text{systolic}} - V_{\text{diastolic}}) / V_{\text{systolic}}$ . Displays state resistance of blood flow distal to the place of measurement.

PI (Pulsatility Index, Gosling index) – pulsation index, indirectly reflecting the state of resistance blood flow.  $PI = (V_{\text{systolic}} - V_{\text{diastolic}}) / V_{\text{mean}}$ . This indicator is more sensitive than RI, because in the calculations V mean is used, which responds sooner on the changes in diameter and vascular tone than V systolic. PI, RI must be analysed together because they signify different properties of blood flow in the artery. Using only one of them can cause diagnostic errors.

Endothelial damage was assessed by determining the number of desquamated endothelial cells (DEC) in the blood plasma of patients. The Micros MCX-100 Daffodil microscope was used for the work. The method of determination was carried out as follows. A positive pressure that exceeds systolic blood pressure by 40 – 50 mm. rt. art. is created in the shoulder area, after 4 minutes a decompression is produced, then venous blood collection is carried out, followed by determination of the number of desquamated endothelial cells. Endothelial damage was assessed by determining the number of desquamated endothelial cells (DEC) in the blood plasma of patients with a value greater than  $2.77 \times 10^5$  in 1 liter of plasma. The method has a high sensitivity and allows to diagnose latent endothelial dysfunction [7]. The Micros MCX-100 Daffodil microscope was used for work.

The results of the work were considered by the commission on bioethics. The study followed the rules of health safety and preserved the rights of the examined children. There were no violations of moral and ethical norms during the research.



**Table 1.** Date of reactive hyperemia test

Date	Before compression n=80	After compression n = 80
Vmax, cm /s	86,8 ± 1,02	104,1 ± 2,16
Vmin, cm /s	0	1,14 ± 0,06
Vm mean, cm /s	9,48 ± 0,59	8,61 ± 0,63
PI, mm	1,00 ± 0,04	4,5 ± 0,27
Ri,mm	5,71 ± 0,69	0,91 ± 0,07
ΔD, %		8,59
IMT, mm	0,56 ± 0,03	0,56 ± 0,03

**Table 2.** Indicators of the reactive hyperemia test in the dynamics of treatment in the children with EAH with ED, M ± m

Date	before compression		after compression	
	Basic therapy n=40	Optimized therapy n=40	Basic therapy n=40	Optimized therapy n=40
Vmax (cm /s)	79,12±2,01	86.80±1,02	95,36±3,12	104.1±2,16
Vmin (cm /s)	0,15±0,01	0	1,02±0,31	1.14±0,06
Vm mean (cm /s)	5,57±0,37	5,48±0,59	7,61±0,42	8.61±0,63*
PI (mm)	8,64±1,78	8,40±0,04	5,31±1,73*	4.52±0,27*
RI (mm)	1,00±0,02	1,00±0,69	0,90±0,11	0.91±0,07
ΔD (%)	0	0	8,86	9,69
IMT (mm)	0,56±0,03	0,55±0,02	0,56±0,03	0,50±0,01

Note. \* p < 0.05 between groups

## RESULTS

The results of outpatient measurements revealed significant differences between systolic blood pressure ( $148.92 \pm 0.74$  mm Hg versus  $109.46 \pm 1.32$  mm Hg,  $p < 0.001$ ) and diastolic blood pressure ( $92.90 \pm 1.78$  mm Hg vs.  $64.11 \pm 2.97$  mm Hg,  $p < 0.001$ ) in the main group and control group. Metabolic changes and increased bioelectrical activity of the left ventricle during ECG examination were also detected. Changes in the morphofunctional characteristics of the left ventricle in the cases of children were insignificant, significant changes were observed between myocardial mass index of the left ventricle (LV MMI), final systolic volume of the left ventricle (LV FSV) compared with children in the control group.

A reactive hyperemia test was performed in the children studied contingent for the endothelial dysfunction detecting. The data is presented in table 1.

The initial diameter of the brachial artery in cases of children of the main groups was significantly larger compared to the control group during the performance of reactive hyperemia test. The increasing in artery diameter ( $\Delta D$ ) was less than 10% in the study group that signifies the presence of endothelial dysfunction [6]. There was also an increasing of *Intima-media* thickness (IMT) ( $0.56 \pm 0.03$  mm), which, cannot exceed  $0.41 \pm 0.004$  mm according to the authors data, in identical cases of healthy children from the same age group [8].

Below the ultrasonograms in the studied contingent are presented (Fig. 1,2)

Consider a set of dynamics indicators which characterize the endothelial dysfunction (table 2).

The initial diameter of the brachial artery in main groups children was significantly larger compared to the control group. The artery diameter increasing after reactive hyperemia test in these groups was less than 10%, which is a criterion of the endothelial dysfunction presence. Evaluation of the study of echostructural properties of the carotid arteries showed that in the cases of children of the main groups there is a significant increasing in the thickness of the intima-media complex. The artery diameter after reactive hyperemia test had tendency to increasing, but only in the cases of children receiving optimized treatment and approached to the reference limit (from 8.59% to 9.69%). At the beginning of the study, there was an increase in intima-media thickness (IMT) ( $0.56 \pm 0.03$  mm), the level of which in the dynamics of treatment decreased more significantly in the group with the optimized method of treatment (from  $0.56 \pm 0.03$  mm to  $0.55 \pm 0.02$  mm and from  $0.56 \pm 0.03$  mm to  $0.50 \pm 0.01$  mm and in relation to the data of the control group  $0.44 \pm 0.02$  mm). The vascular pulsation index (VI) was significantly reduced (by 2 times) in the group of children with optimized treatment, but did not reach the reference upper limit. In the control group of children after the compression test a significant maximum increasing of vessel diameter (up to 11.61%) was revealed, which corresponds to the satisfactory vascular wall elastic characteristics and the endothelial dysfunction absence.

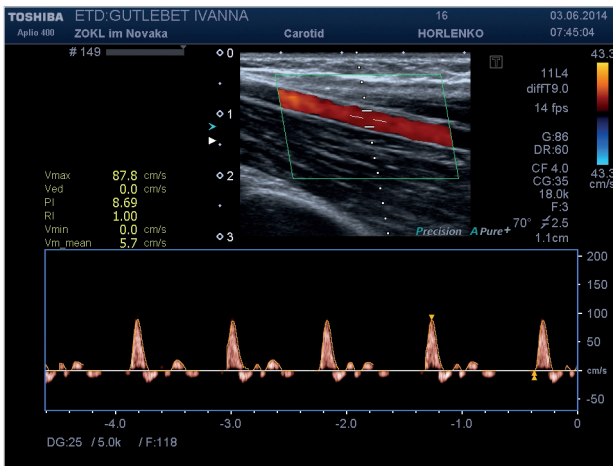


Fig. 1. The state of vascular blood flow before compression

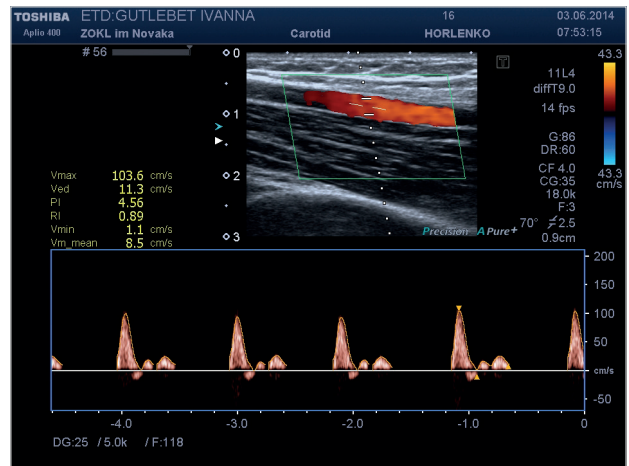


Fig. 2. The state of vascular blood flow after compression

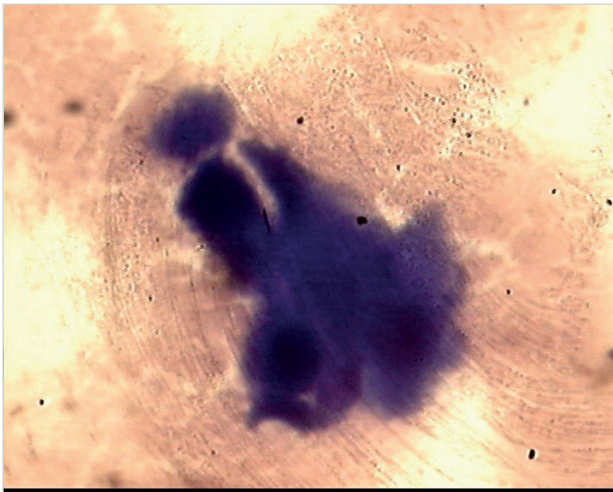


Fig. 3. Indicators of the reactive hyperemia test in the dynamics of treatment in the children with EAH with ED,  $M \pm m$

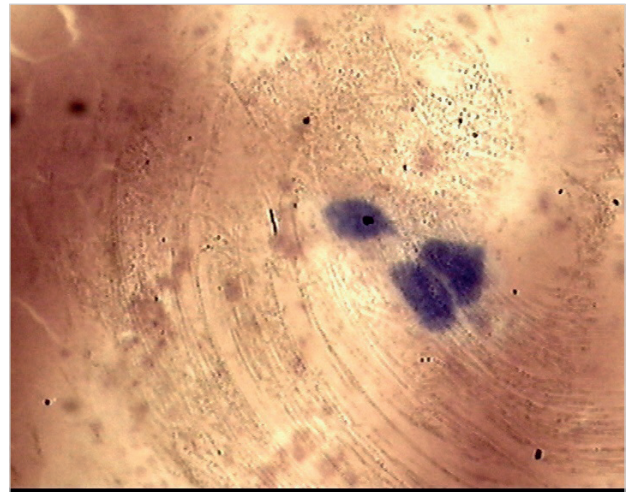


Fig. 4. Complexes of endothelial cells after treatment without signs of polymorphism, the nuclear-cytoplasmic ratio is preserved, fine-grained chromatin. The nucleus is located eccentrically.

Thus, according to our data, hypertension in the cases of children was accompanied by a violation of the elastic properties of the vascular wall.

We performed a dynamic morphometric studies to determine the number of desquamated endotheliocytes (DEC) in the studied contingent (fig.3,4).

The DEC index was in the range of  $3 - 5 \text{ cells} \times 10^5$  in 1 liter of blood ( $4.46 \pm 0.27$  desquamated endothelial cells) according to our data. After treatment,  $2-3 \text{ cells} \times 10^5$  in 1 liter of blood ( $2.28 \pm 0.31$  desquamated endothelial cells) were observed in the field of view, which indicates a positive dynamics of this indicator. The number of desquamated endothelial cells was  $3.56 \pm 0.31 \times 10^5$  in 1 liter of blood in the cases of children using basic therapy. In the control group of children, the process of desquamation of the endothelium is moderate and can be interpreted as a normal physiological process of purification of the intima from damaged cells.

A dynamic echocardiographic study was also performed in the examined pediatric contingent depending on the

treatment method. There is a slight increase in the amplitude of peak E, respectively, in groups and a decrease in the height of peak A in the cases of children of both groups, according to the indicators of transmitral blood flow. The E/A ratio had a tendency to increasing of the levels (from  $1.37 \pm 0.18$  to  $1.60 \pm 0.11$  and up to  $1.80 \pm 0.23$ , respectively, by groups). LV IVRT data tend to decrease (from  $109.32 \pm 9.41$  ms to  $104.44 \pm 8.23$  ms and from  $109.63 \pm 8.39$  ms to  $79.22 \pm 4.41$  ms had and in comparison with data from the control group –  $72.18 \pm 3.41$  ms). That is, the IVRT rate remains prolonged, but in the children group of with optimized treatment it is close to the reference.

Changes in echocardiography parameters: decrease in the peak velocity of the E wave; increasing the peak velocity of wave A; reducing the ratio  $V_e / V_a$ ; prolongation of the isovolumic relaxation phase; the increasing in DT time can be regarded as the initial manifestations of diastolic dysfunction in the examined children. Echocardiography data of study indicators in the examined pediatric contingent

are accorded to the characteristics of the model of diastolic dysfunction (DD) of delayed relaxation. Disturbances in the morphofunctional characteristics of the left ventricle were accompanied by changes in the structure of other cavities of the heart, as well as indicators of the central and peripheral hemodynamics. Confirmation of myocardial remodeling processes in adolescents with EAH can be accompanied by change in such indicators, as LV MMI and relative thickness of the posterior wall of the left ventricle (LV RTPW). These indicators in the studied contingent were higher than in the control, according to our data, which are consistent with the data of many authors.

The aortic stiffness index levels also tended to decrease (from  $0.88 \pm 0.02$  to  $0.71 \pm 0.01$  and to  $0.63 \pm 0.01$ , respectively, by groups and compared with the control group –  $0.55 \pm 0.01$ ), which reflects the improvement of hemodynamic parameters. There was an increasing in the time of isovolumic relaxation of IVRT and a decreasing in the rate of early diastolic filling, which are signs of diastolic dysfunction, according to our data.

LV MMI decreased in the dynamics after the treatment and was  $75.88 \pm 2.43$  g / m<sup>2</sup> and  $73.09 \pm 2.18$  g / m<sup>2</sup>, respectively, by groups, which allows us to make conclusions about the reverse trend of left ventricular hypertrophy (LVH) in patients with EAH. This was expressed in a decreasing of end-diastolic volume (EDV) (from  $108.13 \pm 2.01$  ml to  $98.97 \pm 3.4$  ml in the group with optimized treatment and without changes in the group receiving basic treatment) and absolute values increasing of SV (from  $65.02 \pm 2.73$  ml to  $70.38 \pm 1.78$  ml and from  $65.02 \pm 2.73$  ml to  $80.10 \pm 2.18$  ml, respectively, by groups), EF – from  $65.94 \pm 1.88\%$  to  $69.32 \pm 1.61\%$  and from  $66.27 \pm 1.95\%$  to  $75.00 \pm 1.38\%$ , respectively, by groups). This increasing is characteristic for myocardial hyperkinesia. The vasoconstriction was significantly reduced after the reactive hyperemia test in the cases of patients who have received optimized treatment with lecithin. The obtained data on changes in the profile of LV diastolic filling and arterial endothelial function disorder indicate a positive effect on the total cardiovascular complications risk in the cases of children with EAG in association with ED who have received optimized treatment.

## DISCUSSION

Studies have shown that the problem of endothelial dysfunction has proven clinical significance and requires in-depth study in the cases of children, in recent years, because the detection of this condition and timely elimination of its consequences will prevent more serious diseases in adults.

According to the American Society of Echocardiography, there are four reasons for the IMT of the main arteries: age, professional sports, subclinical atherosclerosis and vasculitis [9]. Our study contingent excludes other risk factors for the development of EAG in association with endothelial dysfunction, in addition to the picture of subclinical atherosclerosis with initial vascular damage. In the control group of children, a significant maximum increase

in vessel diameter (up to 11.61%) was revealed during the compression test, which corresponds to satisfactory elastic characteristics of the vascular wall and the absence of endothelial dysfunction. Thus, Hypertension in the cases of children was accompanied by a violation of the elastic properties of the vascular wall. However, the normal response of the endothelium of the brachial artery was not fully restored. Although, the degree of vasodilation varied depending on the method of therapy. Our correlation analysis did not reveal a connection between the ability of the brachial artery to dilate, the level of blood pressure, heart rate, EDV, EF and indicators of the phase-volume structure of diastole. LVH also did not correlate with Doppler indices and vasodilating activity of the brachial artery. The indicative fact is that endothelial disorders of dependent relaxation and diastolic LV dysfunction are common, on the base of regression of LVH. We can assume that these processes in the cases of patients with EAG exist side by side and independently of each other. But it is possible, that to identify the dynamic effects of endothelial dysfunction on the persistence of phase-volume characteristics of transmitral blood flow and diastolic dysfunction are need for longer-term observation and examination of patients

According to our data, there was an increase in the time of isovolumic relaxation of IVRT and a decrease in the rate of early diastolic filling, which are signs of diastolic dysfunction. There are three models of DD. The first model of DD is called delayed relaxation, or abnormal relaxation, which is hemodynamically represented by a decrease in the volume of blood entering the ventricle in the early filling phase and an increasing in the contribution of the atria. The mitral valve leaflets open later and the duration of the isovolumic relaxation phase increases, as a result of the decreasing in the rate of drop of intraventricular pressure. Slowing of ventricular relaxation leads to an increasing of the volume of blood in the atrium before the onset of systole, which causes an increasing in the force of atrial contraction [10]. Diastolic dysfunction in the studied contingent is a pathogenetic factor that contributes to the development of clinical manifestations in the cases of children without systolic dysfunction (SV – from  $65.02 \pm 2.73$  ml to  $70.38 \pm 1.78$  ml and from  $65.02 \pm 2.73$  ml to  $73.10 \pm 2.18$  ml, respectively, by groups and in comparison with the control group ( $76.01 \pm 1.98$  ml) with normal EF values. Thus, the obtained data about changes in the profile of diastolic filling of the left ventricle and dysfunction of arterial endothelium due to treatment of patients with EAG in association with ED indicate a positive effect of optimized treatment on the total risk of cardiovascular complications.

## CONCLUSIONS

1. The study and the obtained dynamic indicators in the cases of patients with EAH in association with ED, taking into account the developed optimized treatment influence on changes in the profile of LV diastolic filling, arterial endothelial dysfunction confirm the possibility



- of positive correction on the total risk of the appearance and development of cardiovascular complications.
- The blood vessels Vasoconstriction after the reactive hyperemia test was significantly reduced, but the degree of vasodilation varied depending on the method of therapy. The intima-media thickness (IMT) decreased by 1.12 times in the cases of children with an optimized treatment, accompanied decreasing of DEC (by 2 time).
  - There is a dynamic decreasing in the level of left ventricular myocardial mass index (LV MMI), reduction of end-diastolic volume (EDV) and increase in the absolute values of shock volume (SV), EF. The  $V_e / V_a$  ratio had a tendency to increasing due to the inclusion in treatment of lecithin in the cases of children with EAH with ED
  - Levels of the aortic stiffness index had a tendency to decrease (from  $0.88 \pm 0.02$  to  $0.71 \pm 0.01$  and to  $0.63 \pm 0.01$ , respectively, by groups and in comparison with the control group –  $0.55 \pm 0, 01$ ), which reflects the improvement of hemodynamic parameters.

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## Conflict of interest:

The Authors declare no conflict of interest.

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