RELATIONSHIP BETWEEN PROINFLAMMATORY AND ATHEROGENIC MARKERS AND VESSELS REMODELING IN PATIENTS WITH HYPERTENSIVE DISEASE

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Abstract. The article presents clinical data that shows the relationship between proinflammatory markers (IL-22) and atherogenic markers (indices of lipid spectrum) in the formation of structural and functional changes in the carotid arteries, which can be considered as early subclinical markers of atherosclerotic vascular damage in the cohort of patients with comorbidity of hypertension and obesity. Such a combined pathology can be considered a trigger of metabolic events adversely affecting the lipid profile and directing these patients to a high cardiometabolic risk group.

Key words: *atherogenesis, hypertension, interleukin-22, lipid profile, proinflammatory status, remodeling of carotid arteries.*

Introduction. Hypertensive disease (HD) is one of the most common diseases having clinical and social significance. All over the world, the number of HD patients is increasing and today on the average is 44% of the general population, but in some countries, these figures are notably higher and reach 50% [1]. Throughout Europe, Ukraine has the highest mortality rate due to cardiovascular diseases, namely 772.1 cases among men and 440.9 cases among women per 100.000 of people. Circulatory diseases are the most common disorders among Ukrainian population (24.2%). HD ranks first (55.8%) in the structure of blood circulatory system morbidity rate [2].

One of the most frequent and most dangerous comorbid conditions of the HD are obesity and lipid storage disease [3–5]. Early systemic manifestations of HD include carotids remodeling. Immune inflammation is the pathophysiological basis for these pathological conditions. Proinflammatory cytokines, involved in the pathogenesis of obesity, dyslipidemia formation, and architectural distortion of blood vessels, are the mediators of this immune inflammation. Recently, the role of interleukins in the pathogenesis of hypertensive disease and its complications has been actively studied. Thus, there have been studies on the biological effects of interleukine-22 (IL-22) being a member of IL-10 family with proinflammatory properties. IL-22 has various effects and is engaged in many physiological and pathophysiological processes, such as inflammation, tissue regeneration, etc. [6]. IL-22 is a proinflammatory cytokine, a homodimer with a molecular weight of 25 kD belonging to IL-10 family. It is mainly produced by activated Th17, in particular, phenotype memory cells and mast cells, is also produced by monocytes, T- and B- cells, NK cells, congenital lymphoid cells [7]. IL-22 stimulates the production of proinflammatory cytokines in human keratinocytes [8]. However, its role in the cardiovascular disease pathogenesis is poorly studied. That is why it is important to determine and analyze the influence of IL-22 upon elastic arteries remodeling through the example of common carotid (CC) in patients with HD and obesity.

2. Purposes, subjects and methods:

2.1. Purpose – is to study anthropometric parameters, peripheral hemodynamic findings, lipid spectrum, as well as structural and functional changes of the common carotid in HD patients with obesity depending on the level of IL-22 and apolipoprotein B (Apo B).

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2.2. Subjects & Methods.The study involved examination of 84 HD patients (33 men and 51 women) at the age from 41 to 78 years, the median age was 58.0 years.

The HD diagnosis was verified using the recommendations of the European Society of Hypertension (ESH) for the management of arterial hypertension (2013). The excessive weight or obesity was established by calculating the body mass index (BMI) according to the classification of the World Health Organization (WHO, 2006). The abdominal obesity was established if waist measurement (WM) for men was more than 102 cm and for women was more than 88 cm, according to the Ukrainian Association of Cardiology as of 2012 [9].

There were the following exclusion criteria: symptomatic arterial hypertension; thyroid gland pathology; autoimmune diseases; oncology; exacerbation of chronic inflammatory processes or acute inflammatory diseases; acute myocardial infarction or stroke, acute failure of left or right ventricular; traumatic injury of the central nervous system; coexisting mental illness, and diffuse connective tissue diseases.

Blood samples for biochemical and immuno-enzymatic analysis were taken from the cubital vein in the morning on an empty stomach.

Determination of lipid metabolism findings, namely total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDLC) in blood plasma on an empty stomach was made by enzymatic method using standard sets with further calculation of low density lipoprotein cholesterol (LDLC), very low density lipoprotein cholesterol (VLDLC), atherogenicity coefficient (AC) and non-HDLC. The level of Apo B was determined using the immunoenzyme method with Assay Max® Human Apolipoprotein B ELISA Kit.

Determination of the level of IL-22 in blood plasma was carried out by the enzyme-linked immunosorbent assay using the Bender Medsystems® Human IL-22 Platinum ELISA Kit.

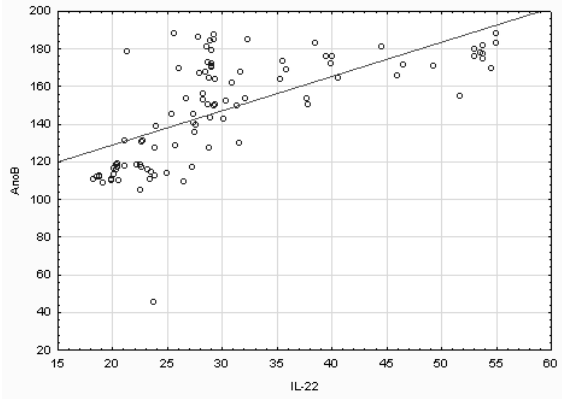
Also the patients underwent an ultrasound investigation of the carotids. The intima-media complex thickness (IMC) was measured in the middle third of the common carotid on the back wall using P. Pignoli method, as the distance between the characteristic echo zone, created by the surfaces of lumen-intima and media-adventitia in the cross-section [10]. The diameter of the common carotid lumen was measured at the same point. The blood flow velocity was also assessed. The relative wall thickness and arterial segment mass were calculated according to the recommendations of the European Society of Cardiology [11]. The common carotid remodeling type was assessed according to the classification offered by O.V. Ahafonov et al. [12].

Statistical analysis of data implied application of nonparametric statistics methods. In samples with nonparametric data distribution, the results were given in the form of Me (Q25; Q75), where Me was median (50th percentile), Q25 and Q75 were 25th and 75th percentile, accordingly. The results were compared by the Mann–Whitney U test. The Spearman correlation analysis was used to measure the degree of dependence. The null hypothesis was refused at the confidence level ($p < 0.05$).

Conflict of interests

There is no conflict of interests.

3. Results and discussion. Comprehensive study of the relationship between IL-22 and Apo B levels and anthropometric parameters, peripheral hemodynamics parameters, lipid metabolism, as well as the thickness of the IMC implied clustering of HD patients into 4 clusters based on the contents of IL-22 and Apo-B in blood serum (*Figure*).



Results of HD patients clustering based on interleukin-22 and apolipoprotein B levels

The first cluster included patients with the lowest IL-22 and Apo B levels, and the second cluster included patients with the highest IL-22 and Apo B levels. The third and the fourth clusters included patients with intermediate IL-22 and Apo B levels.

Characteristics of the first cluster of patients were as follows: levels of systolic blood pressure (SBP) and diastolic blood pressure (DBP), BMI and WM were within normal limits, lipid spectrum findings were within normal limits, the IMC level was also within normal limits (*Table 1*). The second cluster, which included patients with the highest IL-22 and Apo B levels, showed increased

Table 1

General characteristics of hemodynamic, anthropometric, lipid parameters and intima-media complex in clusters

Indices	Cluster 1 N=21	Cluster 2 N=20	Cluster 3 N=23	Cluster 4 N=20
SBP, mm Hg	120 (120; 150)	140 (140; 150)	160 (150; 165)	130 (130; 140)
DBP, mm Hg	80 (80; 90)	80 (80; 90)	100 (90; 100)	80 (80; 80)
BMI, kg/m^2	23 (21; 29)	31 (28; 36)	29 (27; 34)	28 (25; 30)
WM, cm	80 (70; 92)	97 (91.5; 109)	97 (89; 112)	94 (89; 97)
TC, mmol/l	5.2 (4.5; 6.2)	6.67 (5.65; 7.36)	5.80 (4.76; 6.60)	5.97 (4.93; 6.90)
TG, mmol/l	1.87(1.69;2.20)	2.11 (1.85; 2.87)	2.09 (1.75; 2.24)	1.94 (1.56; 2.07)
LDLC, mmol/l	3.01(2.31;3.47)	4.90 (4.64; 5.37)	3.90 (3.75; 4.60)	4.31(2.68; 4.71)
VLDLC, mmol/l	0.85(0.76;1.00)	0.96(0.84; 1.30)	0.95 (0.79; 1.01)	0.88(0.71; 0.94)
non-HDLC	3.8 (2.9; 5.1)	5.4 (4.6; 6.6)	4.65 (3.7; 5.5)	4.65 (3.6; 5.6)
HDLC, mmol/l	1.42(1.09;1.67)	1.05 (0.86; 1.34)	1.05 (0.87;1.26)	1.30(0.93; 1.76)
IMC, mm	0.9 (0.8; 0.9)	1.1 (1.0; 1.15)	1.0 (0.9; 1.1)	1.0 (0.85; 1.0)
D CC, mm	7.1 (6.6; 7.4)	5.8 (5.2; 6.8)	6.0 (5.5; 6.4)	5.7 (5.4; 6.5)

SBP to 140 (140; 150) mm Hg. At normal DBP 80 (80; 90) mm Hg, there was a significant increase in BMI and WM. Lipid metabolism findings in this cluster demonstrated proatherogenic changes, that is why the TC level of 6.67 (5.65; 7.36) mmol/l was above the norm for this cohort, the level of TG of 2.11 (1.85; 2.87) mmol/l was also above the norm, and significantly increased the level of LDLC with median 4.90 (4.64; 5.37) mmol/l, at the same time the level of VLDLC and HDLC of the second cluster patients remained within normal limits.

In the setting of high levels of blood pressure, BMI and WM, and atherogenic dyslipidemia in patients of this cluster there were abnormal findings of IMC 1.1 (1.0, 1.15) mm. IMC changes in the setting of chronic hemodynamic load of high blood pressure and atherogenic dyslipidemia being induced by the improved IL-22 activity (the fundamental for cluster formation) was due to the fact that oxidative stress potentiated the vascular wall inflammation processes, causing increased fibrosis of vessels, proliferation of smooth muscle cells secondary to the reduced endothelium-dependent vasodilation, gradually decreasing elasticity of the arteries and making them stiff. Previous studies have shown the role of proinflammatory cytokines (IL-1, IL-6, IL-17, tumor necrosis factor-α (TNF-α)) in the arterial stiffness pathogenesis [13]. There are also some suggestions that a biologically active TNF-α is produced in response to hemodynamic load created by vascular smooth muscles and endothelial cells. Being a strong inflammatory mediator, TNF-α provides vascular wall hypertrophy and vessels remodeling [14, p. 37].

Assessment of findings in the third and the fourth clusters (with intermediate figures of IL-22 and Apo B) showed the most substantial differences in the third cluster. Patients of this cluster had the highest levels of SBP and DBP, namely 160 (150; 165) and 100 (90; 100) accordingly. Lipid spectrum findings tended to increase above the norm of TC, TG and LDLC, which corresponded to clinical studies, showing that changes in hemodynamic conditions in the case of HD provided vessels remodeling due to the influence upon the vessel walls of blood flow velocity (shear stress), internal pressure of vessels and pressure by the surrounding tissues (transmural stress) [15]. One more study showed that high blood pressure was a triggering mechanism for the development of vessel wall hypertrophy and fundamental for structural arteries change [16]. Lipid storage diseases found in the second and the third clusters in patients with high and intermediate IL-22 and Apo B levels probably reflected additional pathogenic role of lipids in the vessels remodeling, which corresponded to the literature, which showed that IMC changes tended to progress more quickly in dyslipidemia [17, 18].

To better understand the features of vessels remodeling that were peculiar for different clusters, we analyzed the distribution of examined patients by classification of types of geometry of the common carotid according to O.V. Ahafonov (*Table 2*).

Assessment of morphofunctional parameters of the CC showed normal geometry of the CC and the concentric hypertrophy of the CC in the first cluster (*Table 2*). The second cluster was found to have an increase in the proportion of patients with concentric hypertrophy of the common carotid, the third cluster had more patients with concentric CC remodeling, and the fourth cluster had equal number of patients with normal geometry and concentric remodeling.

This cluster analysis showed a possible relationship between the peculiarities of

Table 2

Cluster analysis of the types of geometry of the common carotid

Types of geometry of common carotid arteries	Cluster 1 N=21	Cluster 2 N=20	Cluster 3 N=23	Cluster 4 N=20
Normal geometry CC, absolute (%)	18 (85.7)	6 (30)	7 (30.4)	9 (45)
Concentric remodeling CC, absolute (%)	3 (14.3)	3 (15)	10 (43.5)	7 (35)
Concentric hypertrophy CC, absolute (%)	-	9 (45)	3 (13.05)	3 (15)
Eccentric hypertrophy CC absolute (%)	-	2 (10)	3 (13.05)	1 (5)

distribution of IL-22 and Apo B levels and the nature of structural and functional changes of the CC, in particular the formation of concentric CC hypertrophy and concentric remodeling in patients with the highest levels of hyperinterleukinemia and hypercholesterolemia, which corresponded to a similar clinical study, showing that changes in proinflammatory cytokine IL-33 were associated with CC remodeling and hypertrophy of the vessel wall with mainly concentric variant [19].

The analysis of the correlates of the first cluster with the lowest IL-22 and Apo B levels demonstrated the following IL-22 and BMI relationship ($r = 0.76$; $p < 0.05$), WM ($r = 0.52$; $p < 0.05$), hip width (HW) ($r = 0.52$; $p < 0.05$); TG ($r = 0.44$; $p < 0.05$), HDLC ($r = 0.40$; $p < 0.05$), VLDLC ($r = 0.44$, $p < 0.05$), AC = 0.63; $p < 0.05$), non-HDLC ($r = 0.53$; $p < 0.05$), establishing the relationship between the activity of this cytokine and anthropometric data and changes in lipid metabolism. At the same time positive correlations of IL-22 with the atherogenic pool of lipids and a negative connection with the anti-atherogenic fraction of HDLC attracted more attention.

When analyzing correlations in the second cluster with the highest IL-22 and Apo B levels, the IL-22 level positively correlated with the SBP level ($r = 0.61$; $p < 0.05$), BMI ($r = 0.90$; $p < 0.05$); WM ($r = 0.63$; $p < 0.05$); WM/HW ($r = 0.74$; $p < 0.05$); TC ($r = 0.53$; $p < 0$) , 05%), TG ($r = 0.58$; $p < 0.05$), LDLC ($r = 0.58$; $p < 0.05$), VLDLC ($r = 0.58$; $p < 0.05$), AC ($r = 0.46$; $p < 0.05$) and non-HDLC ($r = 0.58$; $p < 0.05$). In summary, there were similar correlations between anthropometric data and atherogenic pool of lipids; also there was additional relationship between IL-22 and SBP level.

When assessing correlations in the third and the fourth clusters with intermediate IL-22 and Apo B figures no significant differences were found; there continued a similar trend of clearly valid relationship between peripheral hemodynamics data, anthropometric parameters and atherogenic lipid fractions. The third cluster was found to have the relationship between IL-22 and SBP ($r = 0.58$; $p < 0.05$), pulse pressure (PP) ($r = 0.58$; $p < 0.05$), BMI ($r = 0.83$, $p < 0.05$), WM ($r = 0.86$; $p < 0.05$), HW ($r = 0.64$; $p < 0.05$), WM/HW ($r = 0.87$, $p<0.05$), TC ($r=0.74$; $p<0.05$), LDLC ($r=0.75$; $p<0.50$, VLDLC ($r=0.77$; $p<0.05$), CA ($r = 0.78$; $p < 0.05$), non-HDLC ($r = 0.78$; $p < 0.05$). Thus, there was similar relationship; IL-22 also correlated with PP data.

The fourth cluster of IL-22 showed interrelations between IL-22 and BMI ($r = 0.82$; $p < 0.05$); WM ($r = 0.90$; $p < 0.05$); WM/HW ($r = 0.61$; $p < 0.05$), TC ($r = 0.58$; $p < 0.05$), LDLC ($r = 0.61$; $p < 0.05$), CA ($r = 0.58$; $p < 0.05$), non-HDLC ($r = 0.63$, $p < 0.05$).

Thus, all four clusters demonstrated similar clear relationship between IL-22 and blood pressure level, anthropometry, and atherogenic lipid fractions. The relationship between IL-22 and SBP and PP levels was due to the fact that in conditions of hemodynamical "shear stress", occurring in high blood pressure, there was proinflammatory cytokine response to the tissue injury, in which the IL22 was engaged.

The analysis of IL-22 relationship with lipid metabolism figures showed that this proinflammatory cytokine actively correlated with atherogenic lipid spectrum fractions. This analysis allowed to assume that endothelial dysfunction induced by the improved IL-22 activity influenced the increase in the content of adverse lipid fractions and might form a higher potential, which, along with endothelium dysfunction, might ensure the progression of the formation of fatty streaks and further receptor wall damage; this, it its turn, could boost tissue intercellular mechanisms of dystrophy of the smooth muscle layer of the vessel wall.

Assessment of Apo B correlates in the four analyzed clusters showed the following relationship. In the first cluster, Apo B correlated with BMI ($r = 0.72$; $p < 0.05$); in the second cluster, Apo B strongly correlated with BMI ($r = 0.66$; $p < 0.05$); WM ($r = 0.77$; $p < 0.05$); HW ($r = 0.64$; $p < 0.05$); WM/HW ($r = 0.76$; $p < 0.05$); HDLC ($r = -0.58$; $p < 0.05$), AC ($r = 0.45$; $p < 0.05$). The third cluster, having intermediate IL-22 and Apo B findings, had strong relationship between Apo B and BMI ($r = 0.50$; $p < 0.05$), WM ($r = 0.6$; $p < 0.05$), HW ($r = 0.66$; $p < 0.05$), WM/HW ($r = 0.48$; $p < 0.05$), TC ($r = 0.52$; $p < 0.05$), TG ($r = 0.60$; $p < 0.05$), HDLC ($r = -0.47$; $p < 0.05$), LDLC

(r = 0.57; p <0.05), CA (0 , 69), non- HDLC (0.60). In the fourth cluster Apo B correlated with IMT (0.65), MW (r = 0.61; p <0.05), HW (r = 0.51; p <0.05), TC (r = 0, 47; p <0.05), TG (r = 0.58; p <0.05), HDLC (r = -0.56; p <0.05), VLDLC (r = 0.58; p <0.05), AC (r = 0.78; p <0.05), non-HDLC (r = 0.66; p <0.05). General analysis of the Apo B correlates in the clusters being studied showed general positive, valid relationship between Apo B with anthropometric data and atherogenic lipid fractions level. In the intermediate third and fourth clusters, Apo B more strongly correlated with an increased degree of correlation with a greater number of lipid fractions, that is why there were more distinct tendencies of Apo B correlation with non-HDLC, due to active transport intervention of Apo B in the atherogenic processes of blood lipids disorders thus increasing the cardiometabolic risk in patients with HD and obesity triggering additional adverse metabolic lipid disturbances.

The isolated analysis of IL-22 and Apo B activity in the cluster analysis of our study showed clear relationship between IL-22 and anthropometric data and blood pressure levels; Apo B correlated both with anthropometric parameters and atherogenic pool of lipids, however, no relationship between Apo B and peripheral hemodynamics data was found.

4. Conclusions. The cluster analysis revealed a possible relation between the characteristics of the distribution of IL-22 and Apo B level and the nature of structural and functional changes of the common carotid, in particular formation of concentric hypertrophy of the common carotid and concentric remodeling in patients with the highest levels of hyperinterleukinemia and hypercholesterolemia. Based on the clinical features of the common carotid remodeling in HD patients it is possible to assume that IL-22 participates in the development of endothelial dysfunction and atherogenesis in patients with HD and obesity; at the same time Apo B is engaged in lipid disorders, being transported from the increased atherogenic fractions.

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