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INDICATORS OF INFLAMMATION IN THE PATHOGENESIS OF UNSTABLE ANGINA

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ABSTRACT

The aim: Research of blood lipid spectrum, level of anti-inflammatory cytokines and C-reactive protein of coronary heart disease patients.

Materials and methods: There was examined 61 patients with unstable angina, who had been on hospital care in the cardiology department of the Lviv National Emergency Hospital. Their average age was 68.3 ± 1.9 years. The control group included 20 generally healthy persons. There was estimated blood lipid spectrum, C-reactive protein, fibrinogen and proinflammatory cytokine of patients.

Results: There was determined considerable increase total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, triglycerides and coefficient of atherogenicity. High level of C-reactive protein and pro-inflammatory cytokines were detected in patients with unstable angina.

Conclusions: In patients with unstable angina was revealed a significant increase of proinflammatory cytokines levels in the blood serum: interleukin-1 β , interleukin-6, interleukin-17, TNF- α and C-reactive protein, fibrinogen, which indicates activation of the inflammatory process. In patients with unstable angina was detected a significant disorder of blood lipid spectrum. For its correction should be recommended diet and hypolipidemic agents.

KEY WORDS: unstable angina, interleukins, C-reactive protein

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INTRODUCTION

Cardiovascular disease is a leading cause of population disability and mortality worldwide, and angina is the most common symptom of cardiovascular disease [1, 2, 3, 4, 5]. At last count, approximately 9 million people in the United States have coronary heart disease [4]. For the last two decades, ischemic heart disease mortality in Poland increased in people under 65 [3]. Thus, cardiovascular disease is an important medical, biological and social problem as in Ukraine as in other countries of the world. That is why improving diagnostics and strategies for therapy is essential and priority [3, 5, 6]. Pathophysiological basis for the development of cardiovascular diseases, namely coronary heart disease (CHD) is coronary atherosclerosis. The morphological substrate of atherosclerosis is an atherosclerotic plaque, which prevents perfusion in the affected segment, contributes to reduction of coronary blood flow and leads to myocardial ischemia [3, 7].

Significantly increases risk of atherosclerosis at CHD in availability of related risk factors: hypertension, dyslipidemia, smoking, being male, old age, diabetes, increased heart rate, low physical activity, excess body weight, climatic and geographical factors and others. Nowadays, they

number more than 200. Though, smoking 1.6 times more increases the risk of coronary complications, hypertension (systolic arterial vise greater than 195 mmHg) – 3 times, hypercholesterolemia (8.5 mmol / l, 330 mg / dcl or more) – 4 times, the set of three risk factors – 16 times [8, 9].

Unstable angina (UA) constitutes acute coronary syndrome [10, 11]. In unstable angina, the duration of ischemia is insufficient to cause myocardial necrosis. Based on current data, the trigger mechanism of the pathogenesis of unstable angina is damage to the atherosclerotic plaque with thrombosis and episodes of spasm with subsequent progression of stenosis. [3, 7, 12, 13, 14]. An atherosclerotic plaque is formed from an atheromatous (lipid) core and fibroids, which includes smooth muscle cells, collagen, proteoglycans, macrophages, lymphocytes [15]. When an unstable plaque is damaged, after the rupture or erosion of the atherosclerotic plaque, coagulation factors interact with the thrombogenic content of the plaque, specifically collagen, thrombosis are developing. If the balance between prothrombotic and fibrinolytic mechanisms is unfavorable – occlusive blood clots are formed in this section of the vessel crease, which cause acute coronary syndromes, specifically unstable angina. The plaque vulnerability depends on the location, size and

Table I. Risk factors of coronary heart disease in patients with unstable angina

Risk factors	Total patients with UA (n=61)		Male (n=22)		Female (n=39)	
	a6c.	%	a6c.	%	a6c.	%
Age	57	93,4	21	95,5	36	92,3
Heredity	14	22,3	4	19,0	10	25,6
Arterial Hypertension	57	93,4	20	90,9	37	94,9
Hypodynamia	28	45,9	10	45,5	18	46,2
Overweight	12	19,7	5	22,7	7	17,9
Impaired glucose tolerance	12	19,7	3	13,6	9	23,1
Obesity	17	27,9	3	13,6	14	35,9
Dyslipidemia	49	80,3	18	81,8	31	79,5
Smoking	16	26,2	15	68,2	1	2,3
Alcohol consumption	5	8,2	4	18,2	1	2,3
Psycho-emotional factors	21	34,4	7	31,8	14	35,9
Occupational hazards	8	13,1	4	18,2	4	10,3

Table II. Clinical characteristics of patients with unstable angina

Risk factors	Total patients with UA (n=61)		Male (n=22)		Female (n=39)	
	a6c.	%	a6c.	%	a6c.	%
Nature of pain						
Clutching	53	87,0	19	86,4	34	87,2
Scorching	8	13,0	3	13,6	5	12,8
Localization of pain						
Behind the sternum	57	93,4	20	90,9	37	94,9
In the heart area	4	6,6	2	9,1	2	5,1
Irradiation	11	18,0	3	13,6	8	20,5
SBP mm Hg	153,2	-	150,7	-	153,2	-
DBP mm Hg	90,4	-	89,1	-	91,2	-
HR beats / min	84,0	-	88,2	-	102,9	-
Irregular heartbeat	21	31,8	10	45,5	11	28,2
Conduction abnormality	4	6,6	3	13,6	1	2,6
Ejection fraction, %	61,3	-	60,5	-	61,8	-

composition of the lipid nucleus, circular wall tension, the influence of blood flow on the surface of the atherosclerotic plaque (shear stress) [10, 11, 14, 15, 16]. If the balance is favorable, the blood clot is absorbed, at the site of the lesion there is a growth of connective tissue and calcification of the vascular wall, which, in turn, leads to deformation and stenosis of the vascular gap with consequential emergence of symptoms of stable angina. [16]. It should be noted that the process of destabilizing the fibrous coating of the plaque, especially its rupture, is accompanied by activation of local inflammation. Pathomorphological researches indicate the presence of a large number of inflammatory cells, T-lymphocytes, monocytic macrophages on the place of rupture or on the surface of plaque erosion [9, 13, 17].

Endothelial dysfunction has a great role in the process of atherogenesis. It is an important cause of insufficient

vasodilation and spasm of coronary arteries of different caliber [9].

To understand the processes that develop in an atherosclerotic altered vascular wall, it is important to study the markers of inflammation, since inflammatory processes play an important role in the formation and destabilization of atherosclerotic plaque. From inflammatory markers attract a much attention C-reactive protein (CRP), proinflammatory cytokines: interleukin-1 β (IL-1 β) interleukin-6 (IL-6), interleukin-17 (IL-17), necrosis factor of alpha tumors (TNF-alpha) [18, 19, 20, 21].

THE AIM

Investigation of blood lipid spectrum, levels of proinflammatory cytokines and C-reactive protein, fibrinogen in patients with coronary heart disease.

Table III. Indicators of blood lipid spectrum in patients with unstable angina and control group (M±SD)

Indicators	Patients with UA (n=61)	Control group (n=20)
Total CS, mmol / l	6,02±1,14*	4,77±0,98
HDL, mmol / l	1,21±0,27	1,33±0,48
TG, mmol / l	1,96±0,92*	1,30±0,44
LDL, mmol / l	3,91±0,96*	2,85±0,99
VLDL, mmol / l	0,89±0,42*	0,59±0,20
IA	3,95±1,45*	2,59±0,97

* - The credibility of the difference between the indicators of the control group ($p < 0,01$)

Table IV. Indicators of inflammation in patients with unstable angina and control group (M±SD)

Indicators	Patients with UA (n=61)	Control group (n=20)
CRP mg / l	9,81±4,12*	3,92±1,55
IL-1 β , pg / ml	5,90±1,13*	3,20±0,87
IL-6, pg / ml	7,97±2,56*	4,04±1,66
IL-17, pg / ml	15,30±6,02*	8,66±3,11
TNF- α , pg / ml	1,58±0,76*	0,72±0,25
Fibrinogen, g / l	4,47±1,31*	2,86±0,84

* - The credibility of the difference between the indicators of the control group ($p < 0,01$)

MATERIALS AND METHODS

There were examined 61 patients with acute ischemic heart disease, namely with unstable angina (UA), who were hospitalized in the cardiology department of the National Emergency Hospital in Lviv. Among the surveyed were 39 women (64%) and 22 men (36%), their average age was $68,3 \pm 1,9$ years. The diagnosis of the disease and therapeutic tactics were determined in accordance with the orders of the Ministry of Health of Ukraine, as well as in accordance with the recommendations of the European Society of Cardiologists and the American College of Cardiologists on the management of patients with unstable angina. Patients received standard drug treatment, which included anticoagulants, antiplatelet agents, statins, angiotensin-converting enzyme inhibitors, beta-blockers. To eliminate angina attacks, patients were prescribed nitrates. The study did not include patients with severe cardiac, hepatic, and renal failure, cancer, alcoholism, or drug addiction. The control group included 20 practically healthy individuals of age and gender. The study was approved by the local bioethics committee and conducted in accordance with the principles of the Declaration of Helsinki. The patients were given general clinical and instrumental examination methods, they were determined the indicators of proinflammatory cytokines (interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-17 (IL-17), necrosis factor of alpha tumors (TNF- α) and C-reactive protein (CRP), fibrinogen. Examination of patients was performed on entry to the hospital.

The lipid spectrum of the blood was determined by an investigation in blood serum of total cholesterol of (STC), triglycerides (TG), high density lipoprotein cholesterol (HDLC) with using reagents from "Human" (Germany). The content of low density lipoprotein cholesterol (LDLC)

was calculated using the Friedewald formula: $LDLC = STC - HDLC - (TG \times 0,45)$, where $TG \times 0,45$ – quantity of cholesterol in the composition of very low density lipoproteins (QCCVLDL). The coefficient of atherogenicity (CA) was calculated by the formula A.N. Klimov: $IA = STC - QCCVLDL / QCCVLDL$.

Indicators of proinflammatory interleukins were determined in blood serum by enzyme-linked immunosorbent assay using "Vector BEST" test systems (Russia, Novosibirsk) for determination of the content of IL-1 β , IL-6, TNF- α , "Cytokine" (Russia, St. Petersburg) – for determination of the IL-17 according to the instructions, which are attached to the reagent kits. Quantitative determination of C-reactive protein in blood serum was performed by immunoturbidimetric method on an automatic Cobas Integra 400 plus analyzer. Fibrinogen was determined by the Claus method.

Statistical processing of the results was performed using Microsoft Excel and Statistica 6.0 by statistical analysis method by Student's t-criterion determination, data are displayed as $M \pm SD$, where M is the arithmetic average, SD is the average deviation, the difference between the groups was considered significant at $p < 0,05$. The correlation between the various factors was estimated using the Spearman rank correlation coefficient. The difference between the groups was considered significant at $p < 0,05$.

RESULTS

By the results of the research among the risk factors for the emergence of coronary heart disease in patients with unstable angina we found: hypertension, dyslipidemia,

hypodynamia, overweight, obesity, smoking, psycho-emotional factors (Table I). Many patients had a combination of several risk factors, which in turn contributes to the progression of angina. The clinical characteristics of patients are presented in the table (Table II).

By the analysis of the data from patients with unstable angina, was revealed disorders of lipid metabolism. In patients with unstable angina, compared with indicators in the control group (Table III), was revealed an increase in total cholesterol, triglycerides, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, atherogenic ratio, before treatment.

Thus, the level of total cholesterol of patients exceeded the value of the control group by 26.2% ($p < 0.01$). Triglyceride level significantly exceeded the control figures by 50.1% ($p < 0.01$). The level of LDLC in patients significantly exceeded the indicators of the control group by 37.2% ($p < 0.01$), accordingly, QCCVLDL – by 50.8% ($p < 0.01$). The coefficient of atherogenicity significantly exceeded the control value by 52.5% ($p < 0.01$). HDLC in patients was lower compared to the control group. Herewith, HDLC level in patients with unstable angina before treatment less than 1.8 mmol / l (target value for very high-risk patients) was not detected in any patient.

At the analysis of inflammatory markers there was observed their increase compared with the control group (Table IV). In patients with unstable angina pectoris CRP in blood serum significantly exceeded the value of the control group 2.5 times ($p < 0.01$). Fibrinogen in patients with unstable angina significantly exceeded the value of the control group 1.6 times. Cytokines of the early acute-phase IL-1 β reaction in the serum of patients with unstable angina on entry to hospital significantly exceeded the levels of the control group 1.8 times ($p < 0.01$), IL-6, as an indicator of late acute-phase reaction, in serum significantly exceeds the levels of the control group in 2 times ($p < 0.01$). Increase of cytokines levels can be considered as a factor that shows the severity of the disease. Interleukin-17 exceeded indicators of the control group by 1.8 times ($p < 0.01$). Tumor- α necrosis factor exceeded indicators of the control group by 2.2 times ($p < 0.01$).

In the analysis of the relationship of blood lipid spectrum with markers of inflammation in patients with unstable angina was revealed the following correlative relationships: direct relationship of average power between STC and CRP ($r = 0.51$, $p < 0.05$); positive relationship of average strength between STC and IL-1 β ($r = 0.42$, $p < 0.05$); direct relationship of average strength between STC and IL-6 ($r = 0.44$, $p < 0.05$); positive relationship of weak strength between STC and IL-17 ($r = 0.16$, $p > 0.05$); positive relationship of weak strength between STC and TNF- α ($r = 0.19$, $p < 0.05$); positive relationship of moderate strength between STC and fibrinogen ($r = 0.42$, $p < 0.05$); direct relationship of moderate strength between LDLC and CRP ($r = 0.40$, $p < 0.05$); positive relationship of moderate strength between LDLC and IL-6 CRP ($r = 0.35$, $p < 0.05$); direct moderate relationship between LDLC and TNF- α ($r = 0.36$, $p < 0.05$); positive re-

lationship of moderate strength between LDLC and IL-1 β ($r = 0.33$, $p < 0.05$); positive relationship of low strength between LDLC and fibrinogen ($r = 0.26$, $p < 0.05$). The presence of correlational relationships between blood lipid spectrum and inflammation indicators points at their role and involvement in the pathogenesis of unstable angina, and may be a prognostically important factor in coronary heart disease.

DISCUSSION

The study of inflammatory markers is extremely topical for clinical practice, since they are necessary to confirm atherosclerosis or other related pathological conditions of the body: forecasting of the states, that precede serious complications (unstable angina, myocardial infarction), assessment of coronary atherosclerosis, its progression or regression. In our research we discovered a significant increase of proinflammatory cytokines: IL-1 β , IL-6, IL-17, TNF- α and CRP, fibrinogen in the serum of patients with unstable angina to compare with control group. Our results reveal the activation of local and the development of systemic inflammatory process in patients with unstable angina.

Our results are comparative to the results of other authors. Therefore, Hashmi S. In his study observed an increase in proinflammatory cytokines (IL-17, IL-6, IL-8) and heat-sensitive CRP and reduction of proinflammatory interleukin-10 of patients with unstable angina and acute myocardial infarction [22]. In another study, patients with coronary heart disease was noticed an increase in TNF- α , IL-6, and CRP to compare with a group of volunteers. In the same study, was explored the effect of excess TNF- α on monocytes in the blood and concluded the effect of TNF- α excess on monocytes in the blood was studied and there had been concluded that its excess is the main trigger mechanism to development of atherosclerosis and coronary heart disease [23]. In the experimental studies was shown that TNF- α , a proinflammatory cytokine, is involved in destabilizing the atherosclerotic plaque [24].

So, the research of inflammation in the pathogenesis of unstable angina, ischemic heart disease is important as it gives an understanding of the course of inflammatory processes in the body. It would be interesting to make study these indicators during the course of the disease; during treatment with medicines; the effect of drugs on the level of inflammation.

CONCLUSIONS

In patients with unstable angina was revealed a significant increase in serum levels of proinflammatory cytokines: interleukin-1 β , interleukin-6, interleukin-17, TNF- α and C-reactive protein, fibrinogen, which reveals the activation of the inflammatory process. There was revealed a significant disturbance of blood lipid spectrum in patients with unstable angina. For its correction should be recommended diet and hypolipidemic medications.

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