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Markers of Immune Inflammation in the Diagnosis of Coronary Complications

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Abstract.

Objective: To compare the markers of vascular injury and pro-inflammatory cytokines in patients with complications of coronary artery disease in order to determine their diagnostic utility and ability to predict various types of coronary artery disease.

Materials and methods: We enrolled 164 patients with coronary artery disease into the study. Of these, 40 patients had a diagnosis of ST-segment elevation myocardial infarction (STEMI), 36 patients – non-ST-segment elevation myocardial infarction (NSTEMI), 33 patients – unstable angina and 55 patients had various types of chronic coronary artery disease. The control group comprised 30 patients who had arterial hypertension with no signs of coronary artery disease. We measured the levels of TNF-α, IL-1, IL-6 and antibodies to sulfated glycosaminoglycans (GAGs) in all patients with acute coronary syndrome at baseline and during treatment. The levels of cytokines and antibodies to sulfated GAGs in patients with chronic coronary artery disease and in the control group were measured only at baseline.

Results: Patients with acute coronary artery disease such as myocardial infarction (MI) and unstable angina were reported to have a statistically significant increase in the levels of circulating antibodies to sulfated GAGs and TNF-α, IL-1, IL-6 as compared to the patients with chronic coronary artery disease. However, the levels of circulating antibodies to sulfated GAGs and TNF-α, IL-1, IL-6 were found to be higher in patients with chronic coronary artery disease than in the control group. We found a strong positive correlation between the levels of circulating antibodies to sulfated GAGs and IL-6, while no statistically significant correlations were found between the levels of circulating antibodies to GAGs and IL-1 and between circulating antibodies to GAGs and TNF-α. The correlations between the studied parameters in patients with MI were stronger than in those in patients with UA. However, no statistically significant correlations between circulating antibodies to sulfated GAGs and cytokines were found in patients with chronic coronary artery disease. Conventional treatment for acute coronary syndrome resulted in reduced levels of inflammatory markers and antibodies to sulfated GAGs, but these values failed to reach the levels in the control group which comprised patients without coronary artery disease. Elevated levels of pro-inflammatory cytokines were shown to be associated with both myocardial necrosis and unstable angina in which there is myocardial ischemia, being statistically more significant than in the group of patients with chronic coronary artery disease.

Conclusion: Complications of coronary artery disease are associated with elevated levels of circulating pro-inflammatory cytokines and antibodies to the components of connective tissue. A positive correlation between antibody and pro-inflammatory cytokine levels underlies pathogenesis of immune inflammation in acute coronary syndrome. Our findings have applied importance as they can lead to better screening tests for acute coronary syndrome following the onset of unstable angina.

Key words: acute coronary syndrome, inflammation, cytokines, antibodies to sulfated glycosaminoglycans

INTRODUCTION

The incidence of coronary artery disease has grown to epidemic proportions in the 20th and 21st centuries. Acute coronary syndrome (ACS) which refers to a spectrum of clinical presentations ranging from STEMI to NSTEMI or unstable angina (UA) (4) is used to describe a type of coronary artery disease (CAD). So far, early laboratory studies for diagnosing ACS, particularly UA, and differential diagnosis of various types of ACS remain challenging. Diagnostic challenges occur when either clinical presentations or instrumental findings (ECG, echocardiography, and angiography) are unclear due to the blockade of the left leg of the bundle of His, myocardial scarring, electrocardiostimulation, etc. Diagnostic laboratory tests do not always accurately assess cardiac function which can be related to unstable/vulnerable atherosclerotic plaques. It should be noted that no specific biomarkers that offer early detection of acute coronary syndrome, i.e. specific markers of myocardial damage and necrosis, are currently available (5, 8). In the context of coronary artery plaque instability underlying thrombus formation due to plaque inflammation and endothelial dysfunction, there has been growing interest in the role of inflammatory mediators, such as cytokines, in progression of inflammation (2, 4, 6). ACS appears to affect the connective tissue matrix of atherosclerotic plaque, thus contributing to immune responses to connective tissue components, such as GAGs, which form the bulk of the connective tissue (1, 3). Of all non-skeletal and non-cartilaginous tissues, glycosaminoglycans are predominantly distributed in the heart and vessels. Abnormal accumulation of low-density lipoproteins (LDL) in the arterial wall caused by hypercholesterolemia, formation and destabilization of atherosclerotic plaque (erosion, fissures, rupture) can induce depolimerization of proteoglycan complexes resulting in the formation of free glycosaminoglycans (3, 7). Our hypothesis is that an immune response may develop to some components of connective tissue due to inflammation-related plaque activation in acute coronary syndrome, suggesting a relationship between the immune response and the severity of systemic inflammation.

The purpose of the study was to compare serum cytokine (TNF-α, IL-1β, IL-6) profiles and the levels of antibodies to sulfated GAGs in patients with CAD in order to evaluate inflammatory immune response to coronary complications.

MATERIALS AND METHODS

A total of 164 patients with CAD (86 males and 78 females aged 39-87) were included into the study. The patients were allocated to 4 groups: group 1 – 40 patients with STEMI, group 2 – 36 patients with NSTEMI, group 3 – 33 patients with a diagnosis of UA and group 4 included 55 patients with chronic CAD. The chronic CAD group involved patients with a diagnosis of stable angina and post-infarction cardiosclerosis. 36 sex-and-age-adjusted patients without clinical signs of CAD formed the control group. Good Clinical Practice (GCP) vulnerable subjects were excluded from the study. We used BHOK/ESC guidelines for the management of ACS and stable angina.

We measured cytokine (TNF-α, IL-1, IL-6 and antibodies to sulfated GAGs) levels in patients both on admission and at

discharge. The levels of cytokines in patients with chronic CAD and those in the control group were measured only once. Serum concentrations of TNF-α, IL-1, IL-6 were measured using an enzyme-linked immunosorbent assay and commercially available reagents (CJSC «Vector-Best»). To identify serum antibodies to sulfated GAGs, we used an indirect enzyme-linked immunosorbent assay which is based on a novel approach which has been approved by the Volgograd state medical University, Department of Faculty Therapy (Volgograd, Russia). The statistical analysis was carried out using *Statistica 6.0* and *Microsoft Excel – Data Analysis* packages. The results were considered statistically significant for a confidence interval of $p<0.05$.

RESULTS

Table1 summarizes the changes in the levels of antibodies to sulfated GAGs in patients with ACS, CAD and those who presented without any clinical signs of CAD.

The mean levels of antibodies to polysulfated GAGs appeared to be statistically more significant in patients with ACS than in those with chronic CAD or in patients without any signs of CAD ($t=5.12$; $p<0.05$; $t=5.28$; $p<0.05$). Along with this, serum concentrations of antibodies to sulfated GAGs were not significantly different in patients with chronic CAD and those without any signs of CAD. The levels of antibodies to polysulfated GAGs were found to be statistically more significant in all ACS groups as compared to patients with chronic CAD and the control group. The levels of circulating antibodies to sulfated GAGs in the STEMI group were statistically more significant than in patients with chronic CAD ($t=5.65$; $p<0.05$) or in the control group ($t=5.82$; $p<0.05$). It is noteworthy that in patients with MI the highest levels of antibodies to sulfated GAGs were in the STEMI group ($t=2.28$; $p<0.05$). The levels of antibodies to polysulfated GAGs in patients with UA were lower than in patients with NSTEMI and STEMI; however, this difference appeared to be statistically significant only when compared to the STEMI group ($t=1.72$; $p<0.05$; $t=3.35$; $p<0.05$). The levels of antibodies to polysulfated GAGs in patients with UA were statistically more significant than in patients with chronic CAD ($t=2.52$; $p<0.05$) and in the control group ($t=2.74$; $p<0.05$).

Measurements of serum concentrations of TNF-α, IL-1 and IL-6 in patients with ACS and chronic CAD as well as in the control group showed a statistically significant increase of these mediators in patients with ACS ($p < 0.001$). Cytokine levels in the chronic CAD group were significantly lower than in the ACS group at baseline ($p< 0.001$) but their concentration exceeded the norm and the levels in the control group (TNF-α – by 1.5 times, IL-1 – by 1.4 times and IL-6 – by 1.4 times, respectively). The changes in the levels of cytokines in patients with various types of ACS as compared to the control group are presented in Table2.

Elevated levels of TNF-α, IL-1 and IL-6 cytokines were found in all types of ACS (UA, STEMI and NSTEMI). Baseline cytokine levels were the highest in STEMI patients. In NSTEMI patients baseline cytokine levels were lower than in STEMI patients ($p< 0.001$) but significantly higher than those in the control group and the chronic CAD group. Baseline levels of cytokines in patients with UA were lower than in the NSTEMI group ($p< 0.05$) but significantly higher than those in the chronic CAD group and the control group. Low cytokine expression levels ($p< 0.001$) were in all ACS groups at discharge.

Correlations between levels of (TNF-α, IL-1 and IL-6) cytokines and antibodies to GAGs in patients with various types of ACS are presented in Table3.

Table1. Changes in the levels of antibodies to sulfated GAGs in patients with various types of ACS and chronic CAD

Patient group	N (patients)	Symbol	Levels of antibodies to GAGs (absorbance units $*10^{-3}$)	
			On admission	At discharge
ACS	109	M δ m	2.43 0.74 0.05	2.26 0.78 0.08
UA	33	M δ m	2.23 0.52 0.09	2.31 0.45 0.07
NSTEMI	36	M δ m	2.35 0.77 0.11	2.2 0.67 0.11
STEMI	40	M δ m	2.71 0.58 0.09	2.29 0.82 0.10
Chronic CAD	55	M δ m	1.88 0.39 0.06	
Patients without any signs of CAD	37	M δ m	1.85 0.53 0.08	

Table2. Changes in cytokine levels in patients with various types of ACS and chronic CAD

Cytokine		ACS		STEMI		NSTEMI		UA		Chronic CAD	Control group
		On admission	At discharge	On admission	At discharge	On admission	At discharge	On admission	At discharge	On admission	
TNF-α ng\ml	M	37.08	27.05	55.33	35.53	31.68	18.10	19.74	13.06	12.03	8.03
	σ	23.09	17.37	21.92	19.52	18.61	7.61	8.7	4.39	5.82	4.98
	т	2.21	2.01	3.47	3.17	3.1	1.27	1.51	0.76	0.78	0.83
IL-1 ng\ml	M	46.94	35.82	67.2	44.25	41.86	26.93	27.94	16.74	16.88	12.01
	σ	24.75	14.16	19.96	10.74	20.46	11.69	14.02	5.01	6.86	6.36
	т	2.37	1.65	3.16	1.74	3.41	1.95	2.44	0.87	0.92	1.06
IL-6 ng\ml	M	49.58	23.67	76.18	27.17	42.08	19.97	25.52	16.03	14.57	10.42
	σ	32.61	13.94	33.6	16.99	20.53	8.26	13.69	4.57	7.48	7.05
	т	3.12	1.62	5.32	2.76	3.42	1.38	2.38	0.8	1.01	1.17

Table3. Correlation between levels of cytokines and antibodies to sulfated GAGs in patients with various types of ACS and chronic CAD.

Type of ACS	Correlations	On admission	At discharge
ACS	TNF-α /Antibodies to GAGs	0.591*	0.524*
	IL-1/ Antibodies to GAGs	0.505*	0.288
	IL-6/ Antibodies to GAGs	0.704*	0.513*
UA	TNF-α / Antibodies to GAGs	0.388*	0.358*
	IL-1/ Antibodies to GAGs	0.235	0.122
	IL-6/ Antibodies to GAGs	0.567*	0.443*
NSTEMI	TNF-α / Antibodies to GAGs	0.603*	0.478*
	IL-1/ Antibodies to GAGs	0.389*	0.205
	IL-6/ Antibodies to GAGs	0.769*	0.545*
STEMI	TNF-α / Antibodies to GAGs	0.664*	0.611*
	IL-1/ Antibodies to GAGs	0.471*	0.321*
	IL-6/ Antibodies to GAGs	0.713*	0.533*
Chronic CAD	TNF-α / Antibodies to GAGs	0.271	
	IL-1/ Antibodies to GAGs	0.117	
	IL-6/ Antibodies to GAGs	0.303	

**- A statistically significant correlation coefficient, $p < 0.05$*

Our findings show that there is a strong correlation between IL-6 levels and antibodies to GAGs in patients with ACS. The correlations between IL-1 levels and antibodies to GAGs as well as between TNF-α levels and antibodies to GAGs were moderate. The correlation between cytokine levels and antibodies to GAGs in patients with MI was more pronounced than in patients with UA. Patients with UA demonstrated a moderate correlation between IL-6 levels and antibodies to sulfated GAGs. However, at discharge the patients demonstrated a much less pronounced correlation, suggesting significant changes related to positive therapy outcomes. However, low IL-6 expression levels and those of antibodies to sulfated GAGs can also correlate with persistent systemic inflammation. There was no statistically significant correlation between cytokine levels and the levels of antibodies to sulfated GAGs in patients with chronic CAD, suggesting lack of correlation between these two measurements in patients with stable CAD. This could be accounted for by the fact that in this study pro-inflammatory cytokines were involved in atherosclerotic plaque formation. However, the levels of antibodies to sulfated GAGs were slightly increased because the structure of the plaque was intact.

DISCUSSION

We compared the levels of antibodies to sulfated GAGs in patients with various types of ACS and found that all groups of patients with ACS had significantly increased levels of antibodies to polysulfated GAGs as compared to the control group. This can be caused by the inflamed vascular wall, atherosclerotic plaque which has a distinctly attenuated fibrous capsule and connective tissue degradation which are all perceived by immune cells as foreign antigens. Also, we compared the levels of pro-inflammatory cytokines and found that all groups of patients with ACS had significantly increased levels of (TNF-α, IL-1 and IL-6) cytokines as compared to the control group. Cytokine levels tended to decrease during ACS treatment. We suggest that hypercytokinemia can either trigger or result from endothelial dysfunction and atherosclerotic plaque instability. Immunologic disturbances were more pronounced in MI, suggesting the relationship between the severity and extension of the pathological process, on the one hand, and the severity and cytokines levels, on the other hand. Our findings suggest that there is a correlation between elevated cytokine (IL-6) levels and antibodies to sulfated GAGs in patients with all types of ACS. These findings show that both systemic inflammation and formation of antibodies to the components of connective tissue are involved in the same immunopathological mechanism. We assume that connective tissue degradation in the atherosclerotic plaque and the myocardium in patients with MI causes excessive production of autoantibodies. It is now fairly well established that the functional activity of antibody producing cells is determined by IL-6 which transforms B lymphocytes into plasma cells. Antigen stimulation is likely to result in the secretion of cytokines which promotes antibody formation. Also, we suggest that autoantibodies may affect the vascular wall through the formation of immune complexes in the endothelium and local inflammation which cause atherosclerotic plaque instability and enhanced expression of cytokines. During ACS treatment these changes became less pronounced, and the correlation between cytokine levels and those of antibodies was less significant which could reduce inflammation and antibody production and stabilize atherosclerotic plaque. Along with this, moderate hypercytokinemia with no statistically significant levels of antibodies to sulfated GAGs in patients with chronic CAD contributes to the involvement of pro-inflammatory cytokines in the development of CAD prior to atherosclerotic plaque damage. This could explain the immunoinflammatory theory of atherosclerotic lesions.

From a practical point of view, the findings we provide here give a much deeper insight into the pathogenesis of atherosclerosis and its complications. The described cytokine correlations are involved in mechanisms responsible for disease progression. The study provides evidence that severe hypercytokinemia and elevated levels of antibodies to sulfated GAGs are found in patients with ACS and without any signs of myocardial damage, suggesting a pivotal role of pro-inflammatory cytokines and antibodies to sulfated GAGs in the diagnostics of such type of ACS as UA. Our findings open up the possibility of improving laboratory diagnostic testing of ACS. Measurements of cytokine profiles and antibodies to sulfated GAGs can be used prospectively to predict the efficacy of treatment and to identify high risk patients for coronary complications.

REFERENCES

1. Burns, S. A. Dynamic changes in cytokine levels during hospital treatment in patients with various types of coronary syndrome / S. A. Berns, E. Kiprina S., Shmidt E. A., A. Veremeev V., Barabash L. O. // Medical Immunology Journal. Vol.18, N. 1. 2016. P. 33-40.
2. Davydov, S.I. Clinical significance of measuring antibodies to glycosaminoglycans in acute coronary syndrome. Volgograd, 2004- p. 156. A Ph. D. Thesis.
3. Kukharchuk, V.V. Changes in inflammatory response in patients with ACS and patients with stable angina: biochemical and immunologic aspects / Kukharchuk V.V., Zykov K.A., Masenko V.P., E. Nuraliev Yu., Shakhnovich R. M., Yarovaya E. B. // Cardiology Bulletin. Vol.2, N. 2. 2007. P. 48-55.

4. Ostrovsky, O. V. Laboratory markers of myocardial damage in modern cardiology / Ostrovsky O. V., Tkacheva V. V., Ostrovskaya O. V. // Special Edition of Laboratory Journal. N. 2. 2013. P. 31-36.
5. Regino, Yu.I. Dynamic changes in serum inflammation and oxidation biomarkers in patients with ACS / Ragino Yu.I., Kuimov A.D., Polonskaya Ya.V. Kashtanova E. V., Lozhkina N. G. Balabushevich T. A., Eremenko N. E., Negmadzhonov U. N. // Cardiology Journal. N. 2. 2012. P. 22.
6. Christian, M. Biomarkers and acute coronary syndromes: an update/ M. Christian // European Heart Journal. - 2014. - Vol. 35, 38. - P. 552 – 556.
7. Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC) 2017.// European Heart Journal.- 2018.- Vol.39, Issue 2.-P. 119-177. https://doi.org/10.1093/eurheartj/ehx393.
8. Stephen, K. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis / K. Stephen, Sreenivasa R., Shu J. [et al] // European Heart Journal. - 2014. - V. 35. - R. 578 - 589.