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Original Research

Cytokine profile and C-reactive protein level in patients with ischemic heart disease on the background of metabolic syndrome

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Abstract

Background and aims: In this work, it was aimed to study the levels of interleukins 1β, 6, 18, and C-reactive protein (CRP) in the blood serum of patients with ischemic heart disease (IHD). **Material and method:** Clinical and laboratory examination of 120 persons from verified IHD was carried. Out of which 60 patients with IHD without metabolic syndrome (Group 1) and 60 patients with metabolic syndrome [(Group 2) (59 male, 1 female)]. Age of the patients are 55 ± 2 years. The presence of metabolic syndrome was assessed based on the level of triacylglycerol. The obtained laboratory values were compared with the control group, which included 30 healthy individuals. All study subjects were tested for serum levels of IL-1β, IL-6, IL-18, and CRP. **Conclusions:** It was found that metabolic syndrome in patients with coronary artery disease acts as a trigger for the synthesis of several pro-inflammatory cytokines. Statistically significant increase in IL-1β (1.5 times), IL-6 (8.3 times), IL-18 (1.4 times), and CRP (1.3 times) in Group 2 compared to Group 1 of patients indicates the presence of an inflammatory process. Determination of the cytokine status of IHD patients can be a biomarker for the development of an inflammatory response.

Keywords: cytokines, C-reactive protein, ischemic heart disease, metabolic syndrome

Background and aims

Cardiovascular disease is a cause of 67% of deaths in Ukraine and globally. Ischemic heart disease (IHD) is the most common type of heart disease, which causes substantial morbidity and incapacitation in the population. Within this context, the problem of IHD is one of the leading healthcare issues of the 21st century [1, 2].

Ischemic heart disease involves damage to the heart muscle caused by impaired blood flow in the coronary arteries. Myocardial ischemia is synonymous with atherosclerosis. The cause of this condition is the accumulation of atherosclerotic plaques in the vessels due to increased systemic levels of cholesterol (i.e. the development of metabolic syndrome), resulting in narrowing of vascular lumen and inadequate perfusion of various affected organs [1, 3]. The cumulative effect of various components of metabolic syndrome (MS) can be very detrimental since individual risks synergistically augment one another and the overall risk of IHD becomes quite high. Subjects with MS who were 29–79 years of age

and free from any clinical signs of IHD have been often diagnosed with calcification of coronary arteries on their CT scans. Metabolic syndrome includes the disorders accompanied by the development of hypertension (HT), hyperlipidemia, insulin resistance (IR), and abdominal obesity in the patients. Such clinical manifestations are accompanied by the development of immunodeficiency [3, 4]. The increase in plasma levels of non-specific markers of inflammation serves as an indicator of acute-phase responses; moreover, both the amplitude and the nature of this change to some extent depend on the activity of the disease [5]. Potential pro-inflammatory factors include oxidized low-density lipoproteins and pro-inflammatory cytokines [4]. Both pro- and anti-inflammatory cytokines serve as mediators of intercellular interactions; these substances support local inflammation within the atherosclerotic plaque by activating endothelial cells and by inducing the expression of adhesion molecules and the pro-thrombotic activity of the endothelium [4, 6]. The questions regarding the changes in concentrations of novel cardiovascular biomarkers in patients with IHD, which reflect the status of systemic inflammation, remain currently debatable, therefore the studies in this regard are quite promising.

This work aimed to evaluate serum levels of interleukins 1β, 6, 18, and C-reactive protein (CRP) in patients with IHD.

Material and method

Study design and patients

The authors have performed clinical and laboratory assessments in 120 patients with verified IHD, including 60 IHD patients without a metabolic syndrome (30 males and 30 females) – Group 1 and 60 patients with metabolic syndrome (59 males and 1 female) – Group 2. The mean age of the patients were 55 ± 2 years. The presence of metabolic syndrome was assessed by triacylglycerol levels. The resulting laboratory findings were compared with those in the control group, which enrolled 30 virtually healthy volunteers.

Laboratory, anthropometric, and clinical data collection

Fasting venous blood samples (5 mL each) were obtained from all patients. Blood was sampled into BD Vacutainer plastic blood collection tubes with a dual coagulation activator.

All study subjects were tested for serum levels of IL-1β, IL-6, IL-18, and CRP. Assessment of interleukin levels was performed using reagent kits manufactured by VECTOR-BEST Ukraine on a STAT FAX 303 plus analyzer. The levels of CRP were assessed using a CRPLX reagent kit by Roche Diagnostics on a COBAS INTEGRA 400 plus automatic analyzer.

Statistical analysis

Statistical analysis of the data obtained was performed using mathematical methods and STATISTICA 8.0 software package (Statsoft, USA) with subsequent analysis of study results. Basic statistics, such as arithmetic mean (M) and standard error of the mean (m) were calculated. Results in the tables have been provided as M ± m. The differences between arithmetic means were considered significant at $p < 0.05$. Each variable was tested for normal distribution using the Shapiro-Wilk test. Depending on experimental conditions and distribution of data, inter-group differences were assessed using a paired or unpaired t-test and non-parametric Mann-Whitney test [7].

Results

As a result of our study, we found statistically significant differences in serum levels of investigational interleukins in study patients with IHD (Table 1).

Serum levels of IL-1β in patients of Group 1 were within normal. In patients of Group 2, the level of IL-1β was 1.6 times normal and 1.5 times the level in patients of Group 1.

The level of IL-6 in patients of Group 1 was three times lower than the level in the control group and 8.3 times lower than the level in study patients of Group 2. The serum level of IL-6

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Table 1: Serum levels of interleukins 1β, 6, and 18 in patients with IHD.

Parameters	Groups of subjects		
	Control Group (n = 30)	Group 1 (n = 60)	Group 2 (n = 60)
IL-1β (pg/mL)	1.55 ± 0.05	1.67 ± 0.05 p > 0.05	2.57 ± 0.05 p < 0.05 p_1 < 0.05
IL-6 (pg/mL)	2.1 ± 0.05	0.7 ± 0.05 p < 0.05	5.82 ± 0.1 p < 0.05 p_1 < 0.05
IL-18 (pg/mL)	364 ± 5.0	333.4 ± 5.0 p < 0.05	471.7 ± 5.0 p < 0.05 p_1 < 0.05

Note: p = the probability of differences compared to findings in the control group; p_1 = the probability of differences compared to findings in patients of Group 1.

Table 2: Serum levels of C-reactive peptide in patients with IHD.

Parameters	Groups of subjects		
	Control Group (n = 30)	Group 1 (n = 60)	Group 2 (n = 60)
CRP (mg/L)	2.8 ± 0.1	2.99 ± 0.1 p > 0.05	3.85 ± 0.1 p < 0.05 p_1 < 0.05

Note: p = the probability of differences compared to findings in the control group; p_1 = the probability of differences compared to findings in patients of Group 1.

in study patients of Group 2 was 2.8 times higher than the respective level in healthy individuals.

Serum IL-18 level in patients of Group 1 was characterized by the same trends as the levels of previously described cytokines: it was 1.1 times lower than normal and 1.4 times lower than the respective level in Group 2.

As a result of our study, we have found a 1.4-fold increase in CRP levels in study subjects of Group 2 relative to controls (Table 2).

In Group 1, there were no statistically significant differences in CRP levels from the control group; CRP levels in patients of Group 1 were 1.3 times lower than those in patients of Group 2.

Discussion

Therefore, patients with IHD and comorbid metabolic syndrome were found to have substantially elevated levels of investigational interleukins. The presence of metabolic syndrome in such patients leads to the intensification of inflammatory processes, which was manifest as an increased synthesis of pro-inflammatory interleukins.

Pro-inflammatory cytokines (such as IL-1β, IL-6, IL-18, etc.) were produced and act upon immunocompetent cells, thereby initiating inflammatory responses. Many members of the research community [8–10] make a point that increased levels of these cytokines reflect the activity and severity of the disease. The effects of cytokines were closely related to physiological (normal) and pathophysiological responses in the body. With that, there is a modulation of both local and systemic defenses.

IL-1β is a multifunctional cytokine with a wide range of actions. It plays a key role in the development and regulation of general defenses and specific immunity; also, it is one of the first substances to be recruited into the defensive response of the body to various pathogenic factors. Macrophages and monocytes are the principal producers of this cytokine [4].

When influenced by IL-1, endothelial cells of the human vascular system secrete

polypeptides, which are similar to platelet-derived growth factor. These polypeptides may stimulate migration and proliferation of cells and trigger the release of vascular mediators of inflammation; in a setting of significantly increased levels of this cytokine, this may lead to disseminated intravascular coagulation [6, 10].

Both IL-1 and IL-6 initiate the synthesis of acute-phase proteins. Increased levels of IL-1 and IL-6 are associated with repeat coronary events in patients with IHD. Several highly powered studies [11] have shown elevated IL-6 levels to have more important prognostic significance for cardiovascular deaths and other cardiovascular complications compared to C-reactive peptide [5].

Research papers [4, 6, 11, 12] present the evidence of increased IL-2, IL-4, IL-6, IL-12, and IL-18 levels in IHD patients with metabolic disorders as opposed to healthy individuals; along with this, IL-6 levels were even higher in patients with myocardial infarction. IL-6 is one of the multifunctional cytokines, i.e. those that stimulate the proliferation of T-cells, macrophages, and endothelial cells [11]. It also has effects in hematopoietic precursor cells and serves as a growth and differentiation factor for B-cells, hepatocytes, and neurons. IL-6 helps activate endothelial cells, monocytes, and execute pro-coagulant reactions. IL-6 modulates immunological processes, inflammation, proliferation, and apoptosis [4, 11, 13].

Testing for IL-6 levels is playing an important role in the diagnosis of congestive heart failure. Circulating cytokines stimulate the secretion of acute-phase proteins, such as CRP. Under normal conditions, inflammation is very precisely regulated by cytokines, which may have both pro-inflammatory and regulatory properties, thereby inhibiting inflammation. Such regulation is very important in preventing excessive tissue damage. If the causative agent of inflammation is not eliminated, the inflammation may persist and become chronic, which leads to significant tissue damage [5, 13].

Thus, IL-18 is known to be induced by stress signals (of neurogenic or bacterial origin). Some authors [14, 15] argue that stress-induced IL-18 release may enhance the IFN-γ/IL-18 cycle: after the first wave of IL-18-induced synthesis of IFN-γ by lymphocytes, the de novo synthesized IFN-γ, in turn, stimulates monocytes/macrophages, leading to an increase in their activity, which, in part, promotes the formation of IL-18 [16]. Thereby, IL-18 not only stimulates the synthesis of IFN-γ but also modulates its functional activity. The researchers have demonstrated [16, 17] that expression of NK cells also occurs under the influence of IL-18; either independently or through IFN-γ, this interleukin stimulates the initiation of apoptotic processes.

Thus, as seen in our studies, an increase in profiles of cytokines (IL-1, IL-6, and IL-18) was observed in subjects with IHD in a setting of metabolic syndrome. According to worldwide statistics, patients with comorbid metabolic syndrome and stable angina die of IHD twice as often as patients with normal metabolism. Adipose tissue is known to be a source of energy in the body and to have additional endocrine functions. Adipocytes secrete more than 50 biologically active substances, which in turn affect metabolic activity in multiple organs, either directly or indirectly, through neuroendocrine mechanisms, i.e. via pituitary hormones, insulin, and catecholamines [17]. The problem of early detection of coronary disease is very pressing for patients with metabolic syndrome since it defines the possibility of timely correction of symptoms [18, 19]. Assessment of interleukin levels in this patient population will allow for early diagnosis of developing cardiovascular disease.

Conclusions

In patients with IHD, metabolic syndrome serves as a trigger for the synthesis of some pro-inflammatory cytokines. The statistically significant increases in IL-1β (1.5-fold), IL-6 (8.3-fold), IL-18 (1.4-fold), and CRP (1.3-fold), which were observed in IHD patients with metabolic syndrome (as opposed to the group of IHD patients without metabolic disorders) suggest the presence of inflammation. Assessments of cytokine status in patients with IHD are potential biomarkers of a developing inflammatory response.

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Conflict of Interest

The authors declare no conflict of interest.

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