ORIGINAL ARTICLE

Journal of Pre-Clinical and Clinical Research, Vol 2, No 1, 075-079
www.jpccr.eu

Clinical usefulness of determining C-reactive protein and fibrinogen concentrations and lipid profile in blood serum of patients undergoing surgery due to atherosclerosis

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Abstract: Determination of basic blood biochemical parameters are used as a measure taken in atherosclerosis prophylaxis, or is performed depending on the complications found in the course of the disease. The aim of the study was to find differences between basic biochemical parameters measured in blood serum of patients with atherosclerosis. The study comprised 52 men, who were divided into three groups depending upon the type of surgical procedure performed due to atherosclerosis on internal carotid artery, aortic – iliac section and femoral – popliteal section of the femoral artery. The serum concentration of C-reactive protein was determined with the use of the rocket immunoelectrophoresis method (RI). The levels of total cholesterol, its HDL and LDL fractions and triglycerides were measured by the immunoenzymatic method (ELISA). Fibrinogen level was determined according to Clauss's and ELISA tests. Total cholesterol, HDL and LDL fractions and triglycerides remained within the norm. Performed biochemical examinations did not significantly differ between the analysed groups of patients. Collective analysis of the correlation coefficient between the analysed parameters showed that the serum triglyceride concentration decreases with the patients' age. An increase in the acute phase protein concentration was accompanied by a decrease in the concentrations of total cholesterol, LDL and triglycerides. The above relationship was not found in the case of HDL fraction of cholesterol. The results show that the biochemical determinations performed in blood serum of patients with atherosclerosis are not dependent on the location of an obliterated vessel. The significant parameters in this analysis are the acute phase protein concentration (CRP) and the fibrinogen level.

Key words: atherosclerosis, lipid profile, C- reactive protein, fibrinogen, arterial surgery

INTRODUCTION

Atherosclerosis is currently regarded as an active inflammatory process, not as a passive accumulation of lipids, fibrin and extracellular matrix components in the walls of blood vessels, as described in earlier theories. It is a process consisting in intravascular development of a chronic inflammatory condition, resulting from mutual local interactions between modified lipoproteins, macrophages, lymphocytes and thrombocytes, and normal components of the walls of veins and arteries [1]. Many authors also claim that the inflammatory condition in the course of atherosclerosis can be caused by an infection with the Gram-negative bacterium *Chlamydia pneumoniae* [2]. One of the most significant markers of inflammation appearing, among others, in the course of atherosclerosis is C-reactive protein (CRP). This is formed in the liver in response to the development of the inflammatory condition in the organism. There is now an indication that there is a significant relationship between an increase in the plasma CRP concentration and local disturbances in the structure and function of blood vessels.

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Received: 19 April 2008; accepted: 25 May 2008

The phenomenon is more prominent in the case of a simultaneous increase in LDL fraction of cholesterol [3]. It is also claimed that atherosclerosis, dependent on intercellular reactions at the site of endothelial damage, results from stimulation of smooth muscle cells proliferation in vascular walls by cytokines and growth factors released from activated lymphocytes, monocytes and blood platelets [4].

The stimulated muscle cells enable, in turn, accumulation of extracellular matrix proteins at the site of endothelial damage, and formation of the so-called fibrinous atheromatous plaque. Moreover, stimulation of smooth muscle cells increases the transformation of phospholipids and triglycerides, leading to the formation of excessive amounts of cholesterol esters. It has been demonstrated that in vessels changed due to atherosclerosis, as much as 60% of lipids undergo estrification and are deposited in the arterial walls [5].

Low density lipoproteins (LDL), to a large extent, are responsible for the changes occurring in blood vessels. The basic role of LDL is the transport of endo- and exogenous cholesterol. Apart from that, an important role in the transport of lipids to the extracellular space is played by the high density lipoprotein fraction (HDL). It was thus found that disturbances in the relationship between plasma HDL and LDL concentrations significantly contribute to the pathogenesis of atherosclerosis. It has been shown that high LDL or low HDL concentrations are important stimuli in the formation of the atheromatous

plaque. Thus, they are commonly accepted risk factors in the development of atherosclerosis [6].

Plasma triglyceride concentration as a risk factor in the development of degenerative-formative changes in blood vessels has caused a great deal of controversy. The studies conducted so far have not found a definite relationship between the plasma triglyceride level and an increased risk of the atheromatous plaque development. However, analysis of the very low density lipoprotein fraction (VLDL) showed that the plasma triglyceride level can be connected with the early stages of atherosclerosis, regardless of HDL concentration [7].

Despite complicated mutual relationships between LDL, HDL, triglyceride and inflammatory protein levels in plasma, their simultaneous analysis provides important information in the choice of an optimal method of treatment.

The objective of the presented study was to assess the differences or mutual relationships between basic biochemical parameters measured in blood serum of patients with diagnosed atherosclerosis in the aortic-iliac section, femoral-popliteal section and with carotid arteries stenosis. Moreover, the study aimed to assess which of the analysed parameters undergoes the greatest change in the course of the development of atheromatous plaque.

MATERIAL AND METHODS

The study comprised 52 men who underwent operations between the years 1999-2003. The patients were divided into three groups on the basis of physical examination and symptoms, and depending on the type of operation performed.

1. Group I – 13 patients with internal carotid artery potency restored surgically;

2. Group II – 17 patients after the operation for implanting a bifurcated prosthesis due to atherosclerotic obliteration of the aortic-iliac section;

3. Group III – 22 patients after the reconstructive surgery in the femoral-popliteal section due to atherosclerotic obliteration of the femoral artery.

Characteristics

Group I. A tentative diagnosis was made on the basis of history taken and clinical examination performed. All patients had an ultrasound scan taken, with a diagnosis of the internal carotid artery stenosis exceeding 70% of the diameter of the vessel. Next, in order to confirm the results of the examinations, arteriography was performed.

The surgical procedure consisted in restoring the internal carotid artery potency.

Group II. The pain free walking distance of intermittent claudication in the examined group was up to 200 m. Arteriography was performed in all patients. Patients with IVo of ischaemia of the limbs were not qualified for the examinations due to trophic changes which could influence the inflammatory state in the organism (C-reactive protein concentration). The surgical procedure consisted in implanting a bifurcated aortic-bifemoral prosthesis from the intraperitoneal access.

Group III. The analysed group consisted of 7 patients with IIo of limb ischaemia and the intermittent claudication pain free walking distance below 200 m, and 15 patients with IIIo of limb ischaemia who experienced rest pain. Arteriography was performed in all patients before the operative procedure. The reconstructive surgery with the use of a vascular prosthesis was performed in 8 patients, whereas in 14 patients the patient's own vein was used.

During surgical operation, 5,000 units/ml heparin i.v. was administered to all patients. In all analysed patient groups, preventive treatment with antibiotic (Cephazolin – I generation of cephalosporin) was used up to two days after surgery. No medicines influencing CRP or lipids levels during hospitalisation were used.

The follow-up treatment after completion of the therapy was based on daily application of statins, with doses dependent on lipids, and especially on LDL levels.

Blood samples for biochemical analyses were collected from all patients in the presented groups before the surgical procedure.

Laboratory methods

C-reactive protein concentration in blood serum was determined using the rocket immunoelectrophoresis method (RI) modified according to Laurell [8]. Agarose with the addition of appropriate antibodies (anti-CRP) (DAKOPATTS, Denmark) was poured on glass plates. Wells were cut in the agarose after it had set. Two of these wells were filled with solutions of diluted (1:5; 1:10) standards, and the examined sera diluted 1:10 were added to the remaining wells. After placing the plates in the electric field, the moving antigen was bound by the antibodies. Precipitates in the shape of a rocket were formed as a result of the reaction. The planimetrically calculated field of the formed rocket was directly proportional to the concentration of the antigen in the examined serum. The antigen concentration (mg/L) in the analysed sample was interpreted, based on the results of the reactions of standard solutions.

Determination of the lipid profile. Total cholesterol (CH), HDL and LDL fractions and the level of triglycerides were measured using the immunoenzymatic method (ELISA) in accordance with the manufacturer's instructions.

Determination of the fibrinogen level. Determined by the method according to Clauss [9] consisting in assessment of the coagulation time through measurement of changes in the optical density in serum samples after the addition of the thrombin solution.

Determination of the concentration of fibrinogen (mg/dL). Interpreted from the standard curve in the analysed serum sample based on assessment of the coagulation time of different dilutions in standardized serum samples. The determination was performed with the use of the immunoenzymatic technique (ELISA) in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the Fisher-Snedecor method (a method of variance). In order to find whether the mean values of the examined parameters differ between each

other in individual groups of patients; multiple confidence intervals according to Tukey were estimated at p≤ 0.05.

RESULTS

Biochemical examinations performed in blood serum of patients with atherosclerotic arterial obliteration revealed an increase in the fibrinogen level by approximately 50% above the highest value of the accepted norm in all the analysed groups. Moreover, the normal serum C-reactive protein level in the examined groups was exceeded by 8 times. The highest increase in the levels of fibrinogen and the acute phase protein was observed in group II of patients with aortic-iliac obliteration.

The increase, however, was not statistically significant in comparison with the remaining groups of patients (Table 1).

The other biochemical parameters analysed, i.e. the levels of total cholesterol, HDL and LDL fractions and triglycerides, remained within the norm or did not exceed border values of the accepted norms.

Table 1 Acute phase proteins and lipid profile parameters in blood serum of patients with atherosclerosis.

* – significance levels calculated using Tukey test at P-values ≤ 0.05

Parameter	Group of patients	Minimal value	Maximal value	Middle value	Standard deviation
Age	Group I	58	85	68.7	7.3
[years]	Group II	50	83	66.1	8.8
	Group III	51	83	64.7	9.7
	Values of all groups	**50**	**85**	**66.5**	**8.6**
C-reactive	Group I	3	105	34.7	30.4
protein	Group II	2	212	46.7	51.9
[mg/dL]	Group III	2	133	38.7	33.2
	Sum of the groups	**2**	**212**	**40**	**38.5**
Fibrynogen	Group I	366	696	575.2*	79.1
[mg/dL]	Group II	500	1103	657.4	151.7
	Group III	518	898	622.8*	93.4
	Sum of the groups	**366**	**1103**	**618.5**	**108.1**
Cholesterol	Group I	112	285	217.9	48.6
[mg/dL]	Group II	130	312	229.9	45.2
	Group III	113	330	220.6	61.8
	Sum of the groups	**112**	**330**	**222.8**	**51.9**
Cholesterol	Group I	103	194	145.5	30.5
LDL	Group II	67	232	144.5*	41.5
[mg/dL]	Group III	71	260	147.6*	51.8
	Sum of the groups	**67**	**260**	**145.9**	**41.3**
Cholesterol	Group I	26	62	45.2	9.8
HDL	Group II	25	84	50	14.9
[mg/dL]	Group III	33	58	45.6	7.4
	Sum of the groups	**25**	**84**	**46.9**	**10.7**
Triglycerides	Group I	0.53	2.73	1.65	0.77
[mmol/L]	Group II	0.56	2.64	1.55	0.64
	Group III	0.5	2.33	1.35*	0.51
	Sum of the groups	**0.5**	**2.73**	**1.52**	**0.64**

Collective analysis of the correlation coefficient between the examined parameters showed that the serum triglyceride concentration decreases with the patient's age. Apart from that, together with an increase in the acute phase protein concentration, there is a decrease in the total cholesterol, cholesterol LDL fraction and triglyceride concentrations. The relationship stated above was not found in the case of cholesterol HDL fraction (Table 2). In order to confirm that the statistical method chosen for our calculations was right, we analysed the relationship of the total cholesterol concentration and the concentration of its fractions. It was shown that an increase in the serum total cholesterol concentration was accompanied by an increase in the concentrations of HDL and LDL fractions and the triglyceride level. These results confirm that the calculations made were correct, and the choice of the statistical method was right.

Table 2 Correlation coefficient among selected lipid profile parameters performed in blood serum of patients with atherosclerosis. Coefficient is calculated in order to attain a correlation among selected parameters. The coefficient value is contained between -1 to +1. Positive (+) if values of both results are simultaneously increasing or decreasing; negative (-) when one result increases and the second is reduced.

CRP – C-reactive protein, CH – total cholesterol, TG – triglyceride, LDL – low density lipoprotein, HDL – high density lipoprotein.

Parameter	Parameter	Correlation coefficient
Age	TG	- 0.26
CRP	CH	-0.29
CRP	LDL	-0.29
CRP	TG	-0.28
CH	LDL	+0.74
CH	HDL	+0.28
CH	TG	+0.35

In the individual, separate groups of patients, the relationships of the correlation coefficient between the examined blood biochemical parameters were as follows:

Group I patients (atherosclerotic obliteration of the internal carotid artery): an increase in the serum cholesterol HDL fraction concentration was accompanied by a decrease in the fibrinogen level (Table 3).

Table 3 Correlation coefficient among selected lipid profilel parameters performed in serum of patients with atherosclerosis of the internal carotid, aortic-iliac and femoral-popliteal sections.

CRP – C-reactive protein, CH – total cholesterol, TG – triglyceride, LDL – low density lipoprotein, HDL – high density lipoprotein

Parameter	Parameter	Correlation coefficient
Atherosclerosis of internal cartoid		
HDL	FB	-0.56
Atherosclerosis of aortic-iliac section		
CH	LDL	+0.77
CRP	CH	-0.54
CRP	LDL	-0.54
CRP	FB	+0.76
Atherosclerosis of femoral-popliteal section		
CH	LDL	+0.82
CH	TG	+0.70
LDL	TG	+0.54
CRP	FB	+0.61

Group II patients (atherosclerotic obliteration in the aortic-iliac section): an increase in the serum C-reactive protein concentration was accompanied by a decrease in the level of cholesterol LDL fraction. The above relationship did not occur in the case of HDL fraction. Moreover, an increase in the acute phase protein concentration was closely connected with an increase in the fibrinogen concentration (Table 3).

Group III patients (atherosclerotic obliteration in the femoral-popliteal section): in patients with an increasing C-reactive protein level, an increase in the fibrinogen concentration was also found. Moreover, an increase in the total cholesterol concentration was connected with an increasing level of LDL fraction and triglycerides (Table 3).

DISCUSSION

Performing blood serum biochemical examinations in patients with atherosclerotic arterial obliteration not only enables current assessment of the progression of the disease, but the results of analyses of individual parameters should also be treated as independent risk factors for the occurrence of symptoms from the circulatory system.

In the study presented, we also attempted to answer questions concerning relationships between the results of biochemical examinations and the location of atherosclerotic changes, and to find which of the analysed parameters undergo the greatest changes in the course of development of the atheromatous plaque.

After the analysis of the examinations results, no statistically significant relationships were found between the determined biochemical parameters and the location of atherosclerotic changes. This indicates the homogeneity of the atherosclerotic process mechanisms which are not dependent on the site of formation of the atheromatous plaque in the organism.

It was shown in the analyses performed that the mean level of lipids (total cholesterol, cholesterol HDL and LDL fractions and triglycerides) in blood serum of the examined patients did not exceed the accepted norm. This is in accordance with present views that cholesterol is not the real atherogenic stimulus, and that it does not induce pathological changes in the connective tissue of arteries. The lipid deposited in atheromas is cholesterol esters, formed locally by smooth muscle cells of blood vessel walls in response to various stimuli [10, 11]. It is an obvious fact, however, that the ratio of cholesterol HDL to LDL fractions plays an important role in the degenerative-formative processes in blood vessels. In the analysed examinations, the mean HDL level was contained within the normal limits. Although the LDL level exceeded the accepted norm, it remained within the range of border values. This, however, adversely affected the ratio of both fractions, which indicated the high probability of development of pathological changes in arteries. This is in accordance with the results of other authors who have shown that atherosclerotic changes appear more frequently in patients with an elevated plasma level of the low density lipoproteins, with a simultaneous normal or decreased level of the high density fraction [6].

The atherosclerotic process is caused not only by fluctuations in the ratio of HDL to LDL fractions, but also by changes in the vascular endothelium. Endothelial damage induces formation of thrombocyte deposits in damaged areas. Blood platelets aggregate and produce thromboxsan A2 which increases thrombocyte aggregation and induces shrinkage of vessels. This leads to narrowing of the arterial lumen and decreases the flow of blood. Moreover, the aggregating blood platelets produce factors which stimulate smooth muscle cells proliferation in arterial walls. In unfavourable circumstances, for example at high concentration of cholesterol LDL fraction, the atherosclerotic process is stimulated. According to the latest hypotheses, however, repair processes in the damaged endothelium or passive accumulation of lipids in blood vessel walls are not a sufficient explanation for the atherosclerotic process. It is now considered to be an active, chronic inflammatory process, caused by mutual reactions and cooperation of lipoproteins, macrophages, lymphocytes, neutrophils and components of the blood vessel walls structure [1, 4, 12]. The inflammatory process, in turn, results from structural and functional damage to the continuity of the endothelium by stimuli of various origin, and usually lasts for many years. Thus, the atheromatous plaque is formed not only in the areas of irregular anatomical structure or in blood vessels bifurcations, but also in walls of arteries or veins previously weakened by infections or immunologically competent cells in response to the developing inflammatory state [13]. In the conducted examinations, a significant increase in the acute phase protein (CRP) and fibrinogen concentrations in patients with diagnosed atherosclerosis was shown. This confirms the present views indicating that a chronic inflammatory condition and immune mechanisms lie at the basis of the atherosclerotic process progression.

It is indicated that C-reactive protein causes a direct inflammatory effect, regardless of the stage of development of atherosclerosis. Moreover, an increase in the level of this protein stimulates immune competent cells to gather at the site of development of the atheromatous plaque, and to produce pro-inflammatory cytokines which locally aggravate changes in arteries. Thus, C-reactive protein is now considered a marker of not only systemic inflammatory reactions but also an indicator of the progressing atherosclerotic process [1, 3].

The relationship between an increase in the blood CRP concentration and pathophysiology of intravascular thrombi formation is a problem widely discussed at present. We have made an attempt to take part in the discussion, and answer the question about the relationship between the acute phase protein and the fibrinogen level in development of atherosclerosis. As is well known, thrombocytes accumulate at the site of endothelial damage, and a thrombus is formed. The site of endothelial damage, in unfavourable conditions, can also be an area of formation of the atheromatous plaque which is located at the site of an initiated local inflammatory process. This suggests a close relationship between the levels of C-reactive protein and fibrinogen in blood. Moreover, fibrinogen belongs to a group of haemostatic factors which are usually closely related to the inflammatory state, a significant element in initiation and progression of atherosclerosis [14]. In the conducted examinations, the serum CRP concentration in patients with atherosclerosis was found to be, on average. 8 times higher than the accepted norm, and was associated with an approximately 1.5 increase above the upper limit of the norm for fibrinogen. Thus, we confirmed that the level of soluble fibrin precursor is related to a local inflammatory condition and development of, among others, atherosclerosis. A number of studies suggest, however, that an increased fibrinogen level can rather result from the course of atherosclerosis than be its inductor. Other authors, in turn, prove that an

increased fibrinogen level can be a significant risk factor for the development of atherosclerosis and its level in blood; similarly to other haemostatic factors, it can be accepted as a marker of this disease. Unequivocal assessment of the role of fibrinogen in pathophysiology of atherosclerosis development requires further studies [12, 14].

Summing up, atherosclerosis is a multi-factor disease with genetic, environmental and metabolic factors playing a significant role. It is indicated at present that a local inflammatory condition in blood vessels is a stimulus which induces and maintains the development of atherosclerosis. Thus, the analysis of concentrations of inflammatory factors, for example C-reactive protein or pro-inflammatory cytokines, should be a significant parameter of blood biochemical examinations in patients with this disease. Moreover, the atheromatous plaque frequently develops at the site of vascular endothelium damage. It is therefore claimed that haemostatic factors, for example, the level of fibrinogen, can play a significant role in the course of the atherosclerotic process. In the examinations conducted, an increase in the serum C-reactive protein and fibrinogen concentrations was demonstrated in the patients treated. No relationship was found, however, between the serum levels of these factors and the location of the atheromatous plaque in the organism. Therefore, determination of the above-mentioned parameters can complement the examinations of the serum lipoprotein fractions performed so far, and can contribute valuable information in assessing the patient's condition and taking decisions about further treatment.

CONCLUSIONS

1. The results of blood serum biochemical examinations performed in patients with atherosclerosis are not dependent on the location of an obliterated vessel.
2. There are close relationships between the determined parameters, regardless of the location of an obliterated vessel.
3. The levels of C-reactive protein and fibrinogen are of the greatest significance among the determined parameters.

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