Acta Biochimica Polonica

Vol. 46 No. 4/1999

985–989

QUARTERLY

*Communication*

# Elevation of plasma fibrinogen in silent myocardial ischaemia

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Received: 05 July, 1999; accepted: 17 November, 1999

**Key words:** fibrinogen, plasminogen activator inhibitor-1, silent myocardial ischaemia

**High plasma levels of fibrinogen and plasminogen activator inhibitor (PAI-1) are reported to be correlated with coronary heart disease. Therefore the level of fibrinogen concentration in plasma was examined and verified for the possible correlation with the previously explored PAI-1 antigen and PAI-1 activity in the pathogenesis of premature atherosclerosis (Grzywacz *et al.*, 1998, *Blood Coagul Fibrinol.* 9, 245–249). Examination included only men, aged 33-46 years, who were in a stable condition for at least six months after the acute event. They were divided into two subgroups: group A (n = 14) with and group B (n = 15) without ischaemic changes in 24 h Holter electrocardiogram. The number of involved vessels visible on the coronarography picture was similar in both groups. In the patients of group A the mean level of fibrinogen (3.92 *vs* 3.23 g/l, $P < 0.05$) was higher than in the controls (n = 15). No statistically differences were found between group B and control healthy subjects in any of the parameters measured. There were no correlation between fibrinogen concentration and PAI-1 antigen and activity levels, which were elevated in both groups of patients according to our previous study. Our results indicate that elevated levels of plasma fibrinogen and PAI-1 appeared in the group of patients with more severe disease, as revealed by silent myocardial ischaemia.**

The known cardiovascular risk factors, such as hyperlipidaemia, arterial hypertension, diabetes mellitus, obesity and smoking do not entirely explain the pathogenesis of premature atherosclerosis. Nearly 50% of patients suffering from acute myocardial infarction have no identifiable risk factor [1] and even patients sharing similar risk profiles experience varied course of the disease. Recently researches [2] argue for an endothelium damage

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**Abbreviations:** PAI-1, plasminogen activator inhibitor type 1.

as a trigger for interactions between endothelial cells, smooth muscle cells, platelets, macrophages and lymphocytes, leading to the development, growth and remodelling of atherosclerotic lesions in the vessel wall. The disruption of a lipid-rich atherosclerotic plaque participates in the occurrence of arterial thrombosis. The contribution of thrombosis to myocardial ischaemia may be particularly important in young patients with non-severe atherosclerotic lesions or even with a normal coronary picture [3].

Fibrinogen is crucial in determining blood viscosity and coagulation, and it is also an important component of atheromatous plaques [4]. Fibrinogen plays a significant role in two major steps of thrombus formation, i.e. platelet aggregation and fibrin formation [5]. Fibrinogen binds to the platelet glycoprotein IIb/IIIa receptor that leads to aggregation of activated platelets. In fibrin formation, fibrinogen acts as a substrate for polymerization of fibrin monomers. The reduced fibrinolytic activity may depress the rate of removal of intravascular thrombi. This hypofibrinolysis may result from either decreased production or release of tissue plasminogen activator or increased inactivation of this activator by its inhibitor, plasminogen activator inhibitor-1 (PAI-1). Previous studies pointed to fibrinogen as an independent coronary risk factor [6], involved also in the pathogenesis of cerebral stroke and peripheral vascular disease. Other studies have shown association of high plasma PAI-1 level with coronary heart disease [7]. Both fibrinogen level and PAI-1 activity were reported to correlate with standard risk factors for coronary heart disease.

The aim of this study was to assess plasma fibrinogen and verify the possible correlation with PAI-1 levels in young survivors of myocardial infarction, free of coronary risk factors but presenting silent ischaemia.

## MATERIALS AND METHODS

A total of 29 patients, all male, with a history of acute myocardial infarction under the age of 45, and 15 healthy subjects were examined. As a result of 24 h electrocardiogram Holter monitoring the study group was divided into two subgroups: group A (n = 14) with silent myocardial ischaemia and group B (n = 15) without silent myocardial ischaemia. The study was performed for at least 6 months after the acute myocardial infarction and the patients were in a stable phase of coronary disease. All subjects were normotensive, non-diabetic, non-current smokers and with a normal body mass index. We included patients with serum cholesterol levels below 6.5 mmol/l and triglyceride levels below 2.3 mmol/l. The characteristics of both patients and controls was published in detail elsewhere [8].

***Procedures.*** Fibrinogen concentration was measured by the biuret method [9]. Briefly, the fibrinogen was separated from other plasma proteins by clotting the diluted citrated plasma with 5% (0.45 mol/l) calcium chloride. The clot was harvested, washed with normal saline and dissolved with 3% (0.75 mol/l) sodium hydroxide. Subsequently, 20% (0.8 mol/l) cuprum sulphate was added and the absorbance at 530 nm of the supernatant was measured.

PAI-1 activity and antigen were measured in citrated plasma. Blood collection and preparation of platelet-poor plasma for PAI-1 assays were described previously [8].

***Statistical analysis.*** Because of the variation from the Gausian curve, data were expressed as geometrical means and standard deviations (S.D.). Differences between patients and controls, or between subgroups of patients, were evaluated by analysis of variance by the unpaired Kruskal-Wallis test. Correlation analysis of PAI-1 and fibrinogen as well as PAI-1 and lipids was achieved by calcu-

lating the Spearman correlation coefficient. All analyses were performed using the Windows computer program, Statistica™.

## RESULTS AND DISCUSSION

In the patients with silent myocardial ischaemia (group A) we found a higher mean plasma level of fibrinogen as compared to healthy controls (Table 1). The results were compared with those of PAI-1 antigen and activity described in detail for the same group of patients [8]. Moreover, patients in group A exhibited higher PAI-1 antigen levels than those in group B and there were no differences between patients without silent ischaemia and the controls. Fibrinogen level and both PAI-1 antigen and activity were not correlated.

The elevation of plasma fibrinogen and PAI-1 was found in young survivors of myocardial infarction but only in the group of patients with silent ischaemia. Increased levels of these two proteins were reported in coronary artery disease [6, 7] but the population studied here was free of coronary risk factors. Many variables such as age, body mass index, smoking, serum insulin level influence fibrinogen and PAI-1 levels [10]. PAI-1 is affected even more strongly than fibrinogen by metabolic variables as illustrated in the PRIME study [11]. These factors may have influence on the incidence of coronary artery disease. Higher fibrinogen levels at 5 months after the acute coronary event are also predictive of a worse later outcome [12].

The differences in fibrinogen and PAI-1 found in our study could be caused by different reasons. Both fibrinogen and PAI-1 are acute phase reactants. In the course of acute myocardial infarction there is only a transient increase in these two proteins. The present study was performed at least six months after the acute cardiac event and with the patients in the stable phase of the disease. Fibrinogen may be elevated in apparently healthy subjects who are at risk of developing coronary heart disease. The risk is especially high when high plasma fibrinogen concentration is accompanied by a high cholesterol level [13].

The genetic control mediated by environmental factors is the main determinant of plasma protein levels. Recent studies consider several fibrinogen and PAI-1 gene polymorphisms as associated with the development or progression of coronary heart disease. One of the best documented is the G/A sequence variation at position −455 in the promoter region of the $\beta$ chain fibrinogen gene. Carriers of the A allele, representing approximately 20% of the population, have 7–10% higher fibrinogen levels than those with the genotype GG. The fibrinogen A-allele raising effect was greater

**Table 1. Fibrinogen and PAI-1 in patients and controls.**

Means ±S.D. are given.

| Parameter | Patients | | Controls |
|---|---|---|---|
| | group A with silent ischaemia (n = 14) | group B without silent ischaemia (n = 15) | (n = 15) |
| Fibrinogen (g/l) | 3.92 ± 0.68* | 3.55 ± 0.63 | 3.23 ± 0.60 |
| PAI -1 activity§ (U/ml) | 4.90 ± 1.87* | 4.42 ± 2.26 | 3.40 ± 0.97 |
| PAI-1 antigen§ (ng/ml) | 58.1 ± 17.5**# | 41.6 ± 12.6 | 34.8 ± 12.5 |

**$P$< 0.01, *$P$< 0.05 compared to controls; #$P$<0.05 compared to group B; §the results already published [8]

in patients with ischaemic heart disease as compared to healthy subjects [14]. The level of PAI-1 is mainly determined by the 4G/5G polymorphism in the promoter region of PAI-1 gene. Individuals homozygous for the 4G allele, especially myocardial infarction patients, were observed to have increased PAI-1 levels. A higher frequency of the 4G/4G genotype has been reported in young survivors of myocardial infarction compared with age-matched healthy subjects [15]. Seventy percent of our patients had a positive family history of coronary heart disease. This may suggest the influence of genetic factors. In other studies the elevated fibrinogen level was observed in the offsprings of fathers who died at young age of myocardial infarction, pointing to this protein as being a transmissible haemostatic risk factor for coronary heart disease [16].

Another possible reason for the elevation of these two proteins is a chronic inflammatory process within the artery. There is much evidence about the link between atherosclerosis and inflammation [2]. Several cytokines, including interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha and growth factors released during the compensatory response to endothelial injury may stimulate the hepatic synthesis of fibrinogen and PAI-1 [17]. The elevated levels of fibrinogen and PAI-1 still present in our patients 6 months after the acute cardiac event may reflect an ongoing low-grade inflammatory process.

The high fibrinogen level along with high PAI-1 level may represent a thrombotic risk. However we did not find any statistical correlation between those parameters in our patients. It may suggest that altered haemostasis may accelerate the atherosclerosis by different mechanisms. An important finding of our study is the difference in PAI-1 antigen concentration between patients with and without silent myocardial ischaemia despite similar coronarography pictures or metabolic and lifestyle factors. This might point to PAI-1 antigen as a predictor of ischaemic events. Silent myocardial ischaemia present in post-infarction patients was reported to be connected with a worse prognosis [18]. Further investigation is needed to explain whether those patients would benefit from improvement of plasma fibrinolysis. It is also important to focus on patients with high baseline fibrinogen level because pharmacological lowering of plasma fibrinogen may result in a decrease in the occurence of cardiovascular events [19].

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