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Correlation of Plasma Fibrinogen, Plasmin/a2-Anti-Plasmin complex (PAP Complex) and Plasminogen in Non-Smoker and Diabetic Patients with Coronary Artery Disease

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Abstract

Objective: Coronary artery disease (CAD) is one of the major causes of mortality in the world. This indicates the need for identifying biomarkers of CAD diagnosis. Plasminogen as an inactive precursor of the protolithic enzyme plasmin initiates the dissolution of fibrin and consequently lysis of thrombus. Impaired fibrinolysis may cause CAD. This study was aimed to evaluate serum Fibrinogen, Plasmin/a2-Anti-Plasmin complex (PAP complex) and Plasminogen in CAD patients compared to control group.

Materials and Methods: A total of 45 patients with CAD and 45 healthy matched subjects were enrolled. Plasma fibrinogen levels were measured using a commercially available kit according to the Clauss method. PAP and other of parameters were measured using the ELISA, immune enzymatic method according to the manufacturer's instructions (Bioassay technology Laboratory, Instrumentation Laboratory, Shang high, china).

Results: Plasma levels of Fibrinogen were significantly higher in patient groups than control groups. PAP and Plasminogen were significantly lower in patient groups in comparison with controls. The correlations between plasma levels of the markers were not significant.

Conclusion: It is concluded that plasma level of fibrinogen is increased in CAD and plasma levels of plasminogen and plasmin is decreased in CAD, which represent a pathogenic factor for atherosclerosis compared with healthy subjects. Also we recommend the use of these biomarkers as a diagnostic factor for CAD patients. These finding suggested plasma levels of Fibrinogen, PAP and Plasminogen may be useful for early diagnosis of CAD.

Keywords: Fibrinogen, Plasmin/a2-Anti-Plasmin complex, Plasminogen, Coronary Artery Disease, Diabetes, Smoker

Introduction

Despite major progresses in management, coronary artery disease (CAD) remains the biggest cause of mortality and morbidity in the developed world. It is characterized by the activation and aggregation of platelets, thrombus formation and infraction (1). During recent years, many epidemiological studies have been conducted on risk factors of cardiovascular disease including fibrinogen, lipoprotein A, and homocysteine as CAD risk factors. However, it is necessary to assess the new CAD risk factors (2).

Atherosclerosis is a chronic inflammation of arteries in response to the biologic effects of risk factors; it is a qualitative change of endothelial cells when subjected to oxidative, hemodynamic, or biochemical stimuli (due to smoking, hypertension, or dyslipidemia).

They change endothelial permeability to promote the entry and retention of blood-borne monocytes and cholesterol-containing LDL droplets. Inflammation and biochemical changes cause endothelial and smooth-muscle cells to proliferate, produce extracellular matrix molecules, and form a fibrous cover on the newly developed atheromatous lesion (3).

Atherosclerotic plaques (atheroma) are asymmetric focal bump of the inner layer of the artery, the intima. They consist of cells, connective tissue, lipids, and vascular endothelial and smooth-muscle cells.

The atheroma is followed by fatty streak, an accumulation of lipid-laden cells (often macrophages and some T cells) under the endothelium. Many of the immune cells show signs of activation and produce inflammatory cytokines including IL6 and TNFα.

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TNFα stimulates the production of large amounts of acute-phase reactants, including C-reactive protein (CRP), fibrinogen, and serum amyloid A, especially in the liver. These cytokines are also produced in various tissues in response to infection and in the fatty tissue of patients with metabolic syndrome.

Myocardial Infarction (MI) occurs when the atherosclerosis prevents blood to flow through affected coronary artery. Main causes of infarction are due to the formation of an occluding thrombus on the surface of plaque. There are two major cause of coronary thrombus: plaque rupture and endothelial erosion. At these sites, activated immune cells are abundant. They produce numerous inflammatory molecules and proteolytic enzymes that can weaken the cap and activate cells transforming the stable plaque into a vulnerable, unstable structure that can rupture, induce a thrombus, and elicit an acute coronary syndrome (4). A decreased endogenous fibrinolytic system and prothrombotic factors are supposed to influence coronary thrombosis. Impaired fibrinolysis may exacerbate already existing CAD (5). Plasminogen, as the inactive precursor of the proteolytic enzyme plasmin, begins the dissolution of fibrin and cause lysis of thrombus. Conversion of plasminogen to plasmin takes places by activators which are widespread in circulating blood. Plasminogen has a close affinity for fibrinogen and fibrin that this affinity forms part of the thrombosis theory studies showed that clots of low plasminogen content lyse poorly when exposed to activators. Probably a defect in the fibrinolytic enzyme system has a role in causality of thrombolytic disease and consequently fibrin deposition is the leading cause of wall thickening and occlusion (6).

Fibrin may be involved in the tight binding of LDL and accumulation of lipid in the advanced plaque. Epidemiological studies suggest that raised fibrinogen level and clotting activity are related with accelerated atherosclerosis, and although blood fibrinolytic activity has inconsistent results, in arterial intima both fibrinolytic activity and plasminogen concentration are reduced in cardiovascular disease (7). Plasmin is the main enzyme with fibrinolysis action. This action is accelerated by the fibrin and inhibited by PAI-1. Plasmin is rapidly inhibited by a2-antiplasmin, and so the Plasmin/a2-Anti-Plasmin complex (PAP) indicates plasmin generation, and thus fibrinolysis. Elevated PAP level is associated with the incidence of acute MI in the elderly (8), but to our knowledge, there are no data on the correlates of PAP with plasminogen and fibrinogen in non-smoking population.

This study was aimed to evaluate the coagulation and plasma fibrinolysis processes by measuring both PAP-complex and fibrinogen and plasminogen plasma levels in CAD patients and control group; and on the other hand, the study was demonstrated for the first time, correlations between plasma fibrinogen and PAP levels and plasminogen in circulating blood. Whether evaluation and correlation between fibrinogen and fibrinolytic factors is may improve or accelerate methods of CAD diagnosis or not, it may a scientific experiment for future researches.

Material and Methods

An analytical study was conducted on patients aged 40 to 70 years who underwent coronary angiography. A total of 90 subjects, 45 CAD and 45 control, were included into the scope of the study. Venous blood was sampled from non-smoker and diabetic patients with coronary heart disease hospitalized in Shahid Madani Heart Center of Tabriz University of Medical Sciences (TUOMS). Patients who underwent coronary angiography and Per-Cutaneous Intervention (PCI) because of CAD were eligible for the study. Normal venous blood samples were obtained from clinically healthy subjects from Blood Bank of Tabriz. All donors were free of drug therapy. The ethic committee of TUOMS approved the study. Informed consent was obtained from enrolled patients. None of the healthy subjects reported any past history of cardiovascular problems, or had a family history of CAD. Any patient with the history of heart disease, lung disorders, Liver disorders, renal disease and cancer was excluded. In this study, we chose non-smoker individual because smokers have an inflammatory process and may begin acute phase reactant, as previous reports shows that it can elevate plasma levels of fibrinogen (9,10) and may change hemostatic factors.

Venous blood samples were drawn in tubes containing 0.129 mol/l of sodium citrate as anticoagulant. The samples were centrifuged at 3500 rpm for 10 minutes to obtain plasma. The plasma was stored at –80°C until to be assayed.

Plasma fibrinogen levels were measured using a commercially available kit according to the Clauss method. The assay was performed as instructed by the manufacturer (Mahsayaran) in Iran. The assay range is 150-300mg/dl. Plasma plasminogen levels were determined using the ELISA plasminogen assay according to the manufacturer's instructions (Bioassay technology Laboratory, Instrumentation Laboratory, Shanghai, China). The lower limit of detection for the assay is 5%. The intra and inter assay coefficients of variation are 12% and 15%, respectively. In this study we have quantified plasmin activity in blood by measuring the generation of α2-plasmin inhibitor-plasmin complexes that form in clotted plasma. The intra and inter assay coefficients of variation are 10% and 12%, respectively. The assay range is 10-3500 mg/dl. The assay has made it possible to measure resting levels of blood plasmin concentration. PAP and other of parameters were measured using the ELISA, immune enzymatic method according to the manufacturer's instructions (Bioassay technology Laboratory, Instrumentation Laboratory, Shang high, china).

Data are expressed as Mean ± SD or frequency (%). Chi-Square Test was used to analyze categorical data. The SPSS-19 was used to perform all statistical calculations. The significance of differences between groups was assessed by Student's t-test, U Mann Whitney for non-parametric, and correlation among hematologic and plasma protein variables were assessed with Pearson's correlation coefficient. P-Value less than <0.05 was considered statistical significant.

Results

Biochemical analysis of 45 normal and 45 CAD patients were completed (Table 1). Results were shown as means ± standard deviation (SD). Correlations between CAD risk factors were showed in table 2.

Plasma fibrinogen levels were significantly higher in patients with CAD compared to controls (P=0.01). Significant differences were found for plasminogen (P=0.01) and for PAP (P= 0.01) between the two groups (table 1). So, plasma levels of plasminogen and PAP in patients were lower than the controls.

Correlations between CAD risk factors were showed in table 2. The plasma fibrinogen levels showed no significant correlation with plasminogen levels (r=0.02; P>0.05). The plasma fibrinogen levels showed no significant correlation with Plasmin levels (r=0.04; P>0.05), while no significant correlation was observed for the plasma levels of plasminogen and plasmin (r= - 0.08; P>0.05).

Discussion

Atherosclerosis may be documented abruptly when thrombosis occurs on the surface of a disrupted atherosclerotic plaque. This causes symptoms including unstable angina pectoris or MI, if it occurs in the coronary arteries, and transient ischemic attacks or thrombotic stroke in the carotid arteries. Activation of fibrinolytic system is dependent on the conversion of plasminogen to serine proteinase plasmin which splits fibrin to generate soluble degradation products (11-13).

Fibrinogen is a plasma glycoprotein which is converted to fibrin on limited proteolysis by thrombin (10). It represents as an inflammatory marker that appears to be implicated in the pathophysiology and prognosis of CAD. It contributes to the formation of atheromatous plaque via its interaction with other inflammatory substances, endothelium and thrombotic molecules (14). Also it is involved in a number of mechanisms such as endothelial cell injury, platelet aggregation, and plasma viscosity and play a central role in the formation of thrombus (15). However, there is evidence that plasma fibrinogen and other factors are important not only in atherogenesis but also in arterial thrombosis. In addition to these physiological roles, fibrinogen may have a role in the acute and chronic inflammatory process, and an increase in plasma levels of fibrinogen and other inflammation-sensitive proteins is a major component of the acute phase in the chronic inflammatory response (16). Moreover, increased plasma fibrinogen levels are associated with an increased risk of CAD and MI (17).

Table 1: Comparison of biochemical parameters in studied groups

Parameters	Case (n=45)	Control (n=45)	P value
Fibrinogen (ng/dL)	225± 42.3	171± 37.9	0.01
Plasminogen (ng/dL)	76.28± 20.2	87.38± 33.0	0.01
PAP(ng/dL)	1032.65±321.5	1115.43±466.6	0.01

Parameters are shown as mean±SD

Table 2: Pearson's Correlation Coefficients between study variable (in CAD patients)

Parameters	Fibrinogen	Plasminogen	PAP
Fibrinogen (ng/dl)	-	r=0.02 (P=0.83)*	r=0.04 (P=0.67)**
Plasminogen (ng/dl)	-	-	r = - 0.08 (p=0.93)*
PAP (ng/dl)	-	-	-

The Gothenburg Study reported that serum fibrinogen represent an independent risk factor for MI and stroke. Similarly, the Framingham study demonstrated that the risk of MI and stroke rises along with fibrinogen levels. Another large epidemiological study showed that fibrinogen was not only a strong and independent risk factor for MI and sudden cardiac death in patients with previous CAD, but also had a greater predictive value for future coronary events. Despite the data supporting a role of fibrinogen as a marker of CAD and its manifestations, several studies have indicated the role of fibrinogen as a risk factor mediator of CAD. Recently, researchers introduced high levels of fibrinogen as a risk factor for premature CAD in subjects <55 years; also fibrinogen represents an inflammatory marker that appears to be implicated in the pathophysiology and prognosis of CAD (14).

Tatli et al. showed that the association of cardiovascular mortality with fibrinogen levels was independent of established coronary heart disease risk factors and stronger than the association with serum cholesterol (15). Lowe et al. reported that plasma fibrinogen was higher in patients with two or three narrowed coronary arteries than in those with a single affected artery or no stenosis (18). Wilhelmsen et al. reported on the synergistic effect of fibrinogen levels and blood pressure on stroke and suggested that high plasma fibrinogen is a risk factor for stroke and MI (19). Also Acevedo et al. reported that fibrinogen was directly associated with the presence of MI and was revealed to be an independent short-term predictor of mortality (20). Our results are in accordance with the previous results and shows that fibrinogen levels in patients with CAD are increased. Kannel et al. reported elevated fibrinogen level is a predictor of cardiovascular disease that should be added to the cardiovascular risk factor profile (21).

Activation of fibrinolytic system leads to creation of plasmin, a serine protease that cleaves fibrin into soluble fragments (22). Plasmin has a broad substrate specificity and, in addition to fibrin, slices fibrinogen and a variety of plasma proteins and clotting factors (23,24).

Plasminogenis precursor of plasmin and can be activated and converted to plasmin, the active enzyme of fibrinolysis. In addition, plasminogen plays an important role in cell processes (13), and it is suggested that low serum plasminogen may affect fibrinolytic activity and result in thrombotic tendency (23). Booth et al. reported elevated that plasminogen could indicate the inflammation and acute-phase reaction of subclinical atherosclerosis in participants who later developed CAD, because it has been shown that interleukin-6 increases plasminogen transcription. However, they concluded that plasminogen is not correlated with CRP and is correlated only weakly with fibrinogen (24). Meltzer et al. reported that the risk of MI is increased with each increasing quartile of plasminogen (25). In contrast, Smith et al. reported that serum plasminogen is decreased in cardiovascular disease (7).

Besides its role in clot lysis, plasminogen possibly contributes to destabilization of atherosclerotic plaques independent of fibrin proteolysis. Plasmin activates numerous matrix metalloproteinases (MMPs) and it has been reliably shown that MMPs increase atherosclerosis progression and plaque instability (25).

Also, Hoffmeister et al. suggested that plasminogen level is not associated with the incidence of CAD (26). In several large clinical studies, elevated plasma level of both plasmin and its precursor plasminogen were shown to be risk factors for both atherosclerosis and myocardial infarcts.

Although plasmin is the enzyme responsible for fibrinolysis, its production is accelerated by the presence of fibrin and inhibited by PAI-1. Free plasmin is rapidly inhibited by a2-antiplasmin, and the resulting plasmin–a2-antiplasmin complex (PAP) marks plasmin generation, and thus, fibrinolysis. In some studies, elevated levels of PAP are associated with the incidence of acute MI in the elderly, but to our knowledge, there are no data on the correlates of PAP in the general population. Because 80% of fatal MIs occur in older persons, studies of the elderly are needed. PAP had a positive relationship with fibrinogen and CRP and several coagulation factors and thrombin activity, but was a negatively correlated with BMI and PAI-1 as a fibrinolysis inhibitor, while PAP, D-dimer, and plasminogen were positively correlated with each other. The relationship between inflammation and fibrinolysis is complex, and its biology is not completely understood. It has suggested that plasmin generation, as measured by PAP level, is closely associated with ongoing fibrinolysis, subclinical atherosclerosis, and inflammation. Furthermore, PAP levels reflect levels of the major fibrinolysis inhibitor, PAI-1, and increasing levels of PAI-1, and may diminish the relationship observed between plasmin generation and atherosclerosis. Thus, insufficient plasmin generation in presence of a high PAI-1 may be a molecular mechanism for increased thrombosis (8).

The data available on the association between CAD and plasma levels of PAP complexes are few and disagreeing. Increased PAP level is considered as a risk factor for MI and coronary death in a healthy elderly population (27). By contrast, in a small population of survivors of a first MI followed for 2 years, low PAP levels were found associated with an increased risk of re-events (28). Morango et al. reported that higher levels of PAP were associated with an increased risk of cardiovascular death (22).

In this study, results showed that the level of PAP in plasma of CAD patient's was lower than healthy subjects and it may cause a defect fibrinolysis and it was possible to form a thrombus in the arteries.

Our results are consistent with the hypothesis that blood hyper viscosity antedates the onset of symptomatic cerebrovascular/coronary artery disease (29,30).

Conclusion

It is concluded that plasma level of fibrinogen is increased in CAD and plasma levels of plasminogen and plasmin is decreased in CAD, which represent a pathogenic factor for atherosclerosis compared with healthy subjects. Also, we recommend the use of these biomarkers as a diagnostic factor for CAD patients. However, more studies will be required to confirm this hypothesis. We suggest that our findings should be supported by further studies and it should be measured inflammatory markers that participate in atherosclerosis and thrombosis complications. These finding suggested plasma levels of Fibrinogen, PAP and Plasminogen may be useful for early diagnosis of CAD.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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