Plasminogen and fibrinogen plasma levels in coronary artery disease

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Objective: *The formation of thrombi at the site of atherosclerotic lesions plays a central role in atherothrombosis. Impaired fibrinolysis may exacerbate pre-existing coronary artery disease and potentiate its evolution. While the fibrinogen plasma level has been strongly associated with the severity of coronary artery disease, its relevance in the evaluation of plasminogen in coronary artery disease patients remains unclear. This study evaluated fibrinogen and plasminogen levels in subjects with coronary artery disease as diagnosed by angiography.*

Methods: *This is a cross-sectional study. Blood samples obtained from 17 subjects with angiographically normal coronary arteries (controls), 12 with mild/moderate atheromatosis and 28 with severe atheromatosis were evaluated. Plasma plasminogen and fibrinogen levels were measured by chromogenic and coagulometric methods, respectively.*

Results: Fibrinogen levels were significantly higher in the severe atheromatosis group compared to the other groups (p-value < 0.0001). A significant positive correlation was observed between the severity of coronary artery disease and increasing fibrinogen levels (r = 0.50; p-value < 0.0001) and between fibrinogen and plasminogen levels (r = 0.46; p-value < 0.0001). There were no significant differences in the plasminogen levels between groups.

Conclusion: *Plasma fibrinogen, but not plasminogen levels were higher in patients with coronary artery disease compared to angiographically normal subjects. The plasma fibrinogen levels also appear to be associated with the severity of the disease. The results of this study provide no evidence of a significant correlation between plasma plasminogen levels and the progress of coronary stenosis in the study population.*

Keywords: *Plasminogen; Fibrinogen; Coronary artery disease*

Introduction

The formation of thrombi at the site of atherosclerotic lesions plays a central role in the hypothesis of atherothrombosis[1]. A decreased endogenous fibrinolytic system and prothrombotic factors are supposed to influence coronary thrombosis. Impaired fibrinolysis may exacerbate already existing coronary artery disease (CAD) and potentiate its evolution[2].

Several studies have shown that fibrinogen is an important and independent risk factor for CAD[3,4] and this is currently used as a marker of inflammation[4,5]. The increase in the plasma fibrinogen concentration is related to the development of CAD through changes in the mechanisms of platelet aggregation due to the influence of plasma fibrinogen on the quantity of fibrin formed and accumulated and its connection with the evolution of atherosclerotic plaque[6,7] and with the increase in blood viscosity related to the risk of thrombosis[8].

Plasminogen, a zymogen that is usually present in plasma[9,10], is a single-chain glycoprotein synthesized in the liver; it is considered an inactive proenzyme that is converted to plasmin[11]. This serine protease degrades fibrin through the action of physiological activators, including tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), secreted by endothelial cells[12]. The endothelium itself controls the production of these activators through the synthesis of specific inhibitors of the fibrinolytic system, such as type 1-plasminogen activator inhibitor (PAI-1)[12,13]. Some researchers have shown an independent and unexpected association between high plasminogen levels and the risk of CAD[14,15]. However, the relevance of evaluating plasminogen levels in CAD patients remains unclear.

This study aimed to investigate the association between both fibrinogen and plasminogen plasma levels and the presence of increasing degrees of coronary atheromatosis in subjects submitted to coronary angiography.

Methods

Patients

Fifty-seven subjects, with ages ranging from 40 to 65 years, who had been submitted to coronary angiography in the Department of Hemodynamics of the Hospital Socor, Belo Horizonte, Brazil were enrolled in the study. Blood samples were collected before coronary

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angiography. Based on results of the coronary angiography, the participants were classified into three groups: angiographically normal arteries (n = 17), mild/moderate atheromatosis (n = 12) and severe atheromatosis (n = 28).

All patients were referred for cardiac catheterization due to chest pain and/or the presence of demographic profiles and risk factors for CAD. All patients had a history of stable angina. Even so, no patients had had recent myocardial infarction or unstable angina (within the three months preceding blood collection), congestive heart failure, coagulation disorders, renal problems, hepatic or auto-immune diseases or cancer or were on warfarin treatment or undergoing lipid-modifying therapy.

The protocol of this study was approved by the Research Ethics Committees of both of the Hospital Socor (#05/04) and the Universidade Federal de Minas Gerais (UFMG - #137/04), Belo Horizonte, Brazil. The subjects enrolled in this study were informed about the aims of the investigation and those who agreed signed informed consent forms.

Angiography

Coronary angiography was performed by the percutaneous transfemoral approach. The images were recorded digitally and all angiograms were analyzed by three experienced cardiologists. The extent of angiographically documented CAD was quantified as follows: angiographically normal coronary arteries (no stenosis), mild disease (stenosis of up to 30% of the luminal diameter in one or more coronary arteries), moderate disease (stenosis of 31% to 70%) or severe disease (stenosis of more than 70%).

Blood collection and laboratory tests

Venous blood samples were drawn after 12 hours of fasting in vacuum tubes containing 3.2 w/v sodium citrate as anticoagulant. Blood samples were centrifuged at 2500 rpm for 10 minutes to obtain plasma.

Plasma fibrinogen levels were measured using a commercially available kit (Fibrinogen "O") according to the Clauss method. The assay was performed as instructed by the manufacturer (DiaMed® AG, Cressier Sur Morat, Switzerland) in a ST4BIO Coagulometer device (Stago® Diagnostica, Asnieres-sur-Seine, France). The lower limit of detection by the assay is 45 ng/mL. The intra and inter assay coefficients of variation are 2.9% and 6.8%, respectively.

Plasma plasminogen levels were determined using the electrochrome™ plasminogen assay according to the manufacturer's instructions (Hemoliance® Instrumentation Laboratory, Lexington, USA). The lower limit of detection for the assay is 5%. The intra and inter assay coefficients of variation are 1.9% and 4.1%, respectively. For both fibrinogen and plasminogen, no significant cross-reactivity or interference from other coagulation factors have been observed for the assays and commercial control-plasma was used to validate the assay.

Statistical analysis

The sample size was defined using published coefficients of variation for the studied parameters, considering a 10% variation from the average and two degrees of freedom, using a minimum of eleven subjects in each group so that possible statistical differences could be demonstrated with a significance level of 5%.

Data presented normal distribution and were analyzed using the Sigma Stat version 1.0 software system with one-way analysis of variance followed by Tukey's test. Pearson's correlation coefficient was used to measure the linear association between plasma fibrinogen and plasminogen levels, and Spearman's correlation coefficient was used to measure the linear association between CAD and continuous variables.

Results

The three study groups showed homogeneity in relation to age, gender and body mass index (BMI). Results are shown as means ± standard deviation (SD) in Table 1 and graphically in Figures 1 and 2. Plasma fibrinogen levels were significantly higher in patients with severe atheromatosis compared to those with mild/moderate atheromatosis (p-value < 0.0001) or controls (p-value < 0.0001). The difference between controls and the mild/moderate atheromatosis group was also significant (p-value < 0.0001). For plasminogen, significant differences were not found for mean values between the three groups.

Table 1 - Characterization of the studied groups and the biomarker assays results

Parameter	Control	Mild/moderate atheromatosis	Severe atheromatosis	p-value
Gender m/f	8/9	8/4	16/12	
Age (years)	58.9 ± 7.7	61.0 ± 11.6	60.9 ± 9.6	0.515
Body mass index (Kg/m^2)	24.9 ± 4.6	26.1 ± 4.9	24.3 ± 3.2	0.231
Smokers - n (%)	3 (17.6)	3 (25.0)	9 (32.1)	0.432
Arterial hypertension – n (%)	14 (82.4)	9 (75.0)	24 (85.7)	0.842
Sedentary lifestyle – n (%)	16 (94.1)	9 (75.0)	21 (75.0)	0.501
Family history – n (%)	7 (41.2)	5 (41.7)	12 (42.9)	0.347
Fibrinogen (mg/dL)	177.1 ± 36.7	210.9 ± 41.0[a]	243.4 ± 45.1[a,b]	< 0.0001[a,b]
Plasminogen - %	100.1 ± 13.9	96.8 ± 14.1	97.3 ± 12.9	0.622

Data are shown as means ± standard deviation or percentages. Significant differences between the groups are indicated by the letters: a. versus control and b. versus mild/moderate atheromatosis.

The plasma fibrinogen levels showed a significant positive correlation with plasminogen levels (r = 0.46; p-value < 0.0001). The presence of CAD was associated with elevated plasma fibrinogen levels (r = 0.50; p-value < 0.0001), while no association was observed for the other variables.

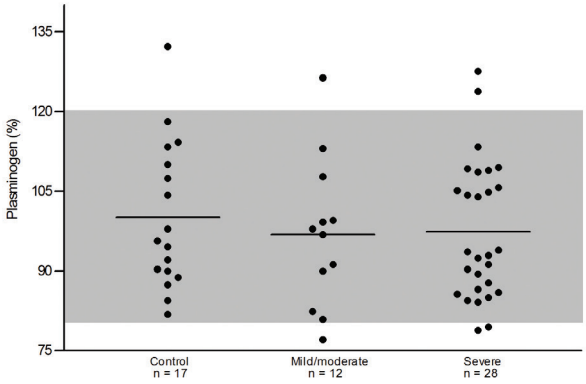

Figure 1 - Plasma plasminogen levels in control (angiographically normal arteries), mild/moderate and severe atheromatosis groups. No significant differences were found between the three groups. The shaded area corresponds to the normal range (80 – 120%) for plasminogen levels; horizontal lines represent the mean values of groups.

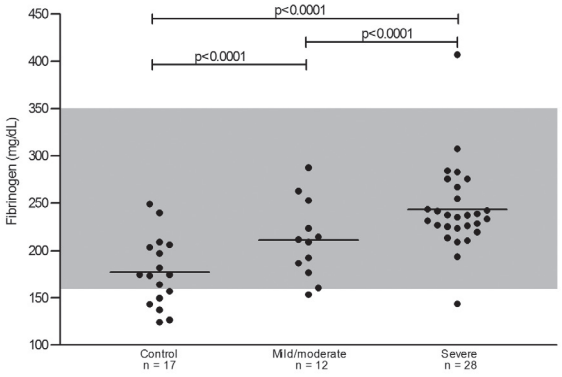

Figure 2 - Distribution of plasma fibrinogen levels in control (angiographically normal arteries), mild/moderate and severe atheromatosis groups. Statistically significant differences were found between the three groups (p-value < 0.05). The shaded area corresponds to the normal range (160 – 350 mg/dL) for fibrinogen levels; horizontal lines represent the mean values of groups.

Discussion

There are several systemic changes in hemostasis and fibrinolysis in atherosclerosis[16]. In this study, the association between the degree of coronary atheromatosis and two key components of the coagulation system were evaluated. Once a thrombus resulting from a hypercoagulability condition is formed it is dissolved by the fibrinolytic system. So, researchers have hypothesized that a decrease in fibrinolytic activity could be a risk factor for ischemic events[17].

It is still unclear in the literature whether the quantity of plasminogen that is transformed into plasmin is linearly correlated to plasminogen levels. Plasminogen plasma concentration does not vary significantly in normal blood coagulation[18]. In the present study, the average values obtained for plasminogen did not show statistically significant differences between the three groups (Table 1) and remained within the normal range indicated for the method. An independent and unexpected association between high plasminogen levels and CAD risk has been reported previously[4,8], since decreased plasminogen levels suggest the generation of less plasmin and signal impaired fibrinolytic activity, which favors the deposit of fibrin and contributes to atherothrombosis. However, plasminogen has several non-fibrinolytic roles the most important of which is related to the inflammatory process[18]. Plasminogen is directly linked to monocyte recruitment during inflammatory response[4] and there is evidence that interleukin-6 may increase the plasminogen gene expression[19]. So, it is likely that the increase in plasminogen plasma concentration is secondary to the inflammatory process present in subclinical atherosclerotic lesions[19]. This may, at least in part, justify the presence of the positive correlation found in this study between plasminogen and fibrinogen levels (r = 0.46; p-value < 0.0001).

Few studies have shown the participation of plasminogen in the local process of atherothrombosis[4,18]. Likewise, in the present study, plasminogen levels were not correlated to the presence or severity of CAD and did not bring additional benefits, at least for subjects with the diagnosis of CAD. Similar observations were made by Hoffmeister et al.[20] in a case-control study; the authors failed to find any association between elevated levels of plasminogen and stable CAD in German patients.

Some systemic inflammation markers may indicate the severity of the inflammatory process and their levels were recently associated to CAD[4,21]. Table 1 indicates that for plasma fibrinogen levels a significant difference was found between the three groups (p-value < 0.0001). Figure 1 shows that the plasma level of fibrinogen increases as coronary atherosclerosis establishes and progresses to the mild/moderate and severe atheromatosis degrees, even though the mean values of groups remained within the normal range for the method. Similar results were reported by Giannitsis et al.[22] who found that the mean values for the plasma levels of fibrinogen increased progressively as the CAD became more severe, although these values remained within the normal range. This current study also showed that, despite being within the normal range, plasma fibrinogen levels are significantly higher in patients with mild/moderate and severe atheromatosis than in angiographically normal subjects. The association between hyperfibrinogenemia and the progression of CAD has been widely reported[8,23-25]. It is well-known that hyperfibrinogenemia is associated to acute coronary events and may be partly a cause and not just a consequence of the lesion as fibrinogen is a protein that appears in the acute phase and as the atherosclerotic process presents inflammatory components[8].

As this is a cross-sectional study, we evaluated associations, not predictions nor causation. Another important limitation is the fact that our observations are based on a small group of patients; further studies involving larger numbers of patients may elucidate the association between plasma levels of plasminogen and the severity of coronary stenosis. Our findings relate to a group of patients that, according to cardiologists, require coronary angiography and thus our subjects with no coronary stenosis may not be representative of the general population.

Conclusion

Plasma fibrinogen, but not plasminogen levels were higher in patients with CAD compared to angiographically normal subjects. The plasma fibrinogen levels also appear to be associated with the severity of the disease. These results give no evidence that there is a significant correlation between plasma plasminogen levels and the progress of coronary stenosis.

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