PAI-1 4G/5G Polymorphism and Plasma Levels Association in Patients with Coronary Artery Disease

Luciana Moreira Lima1,2, Maria das Graças Carvalho2, Cirilo Pereira Fonseca Neto3, José Carlos Faria Garcia3, Marinez Oliveira Sousa2
Departamento de Medicina e Enfermagem - Universidade Federal de Viçosa1, Viçosa, MG; Departamento de Análises Clínicas e Toxicológicas - Universidade Federal de Minas Gerais2; Departamento de Hemodinâmica - Hospital Socor3, Belo Horizonte, MG, Brazil

Abstract

Background: Type-1 plasminogen activator inhibitor (PAI-1) 4G/5G polymorphism may influence the PAI-1 expression. High plasma levels of PAI-1 are associated with coronary artery disease (CAD).

Objective: This study investigated the influence of PAI-1 4G/5G polymorphism on plasma PAI-1 levels and its association with CAD assessed by coronary angiography.

Methods: Blood sample of 35 individuals with angiographically normal coronary arteries, 31 individuals presenting mild/moderate atheromatosis, 57 individuals presenting severe atheromatosis and 38 healthy individuals (controls) were evaluated. In patients and controls, the PAI-1 4G/5G polymorphism was determined by PCR amplification using allele-specific primers. Plasma PAI-1 levels were quantified by ELISA assay (American Diagnostica).

Results: No difference was found between groups regarding age, gender and body mass index. Plasma PAI-1 levels and 4G/4G genotype frequency were significantly higher in the severe atheromatosis group compared to the other groups (p<0.001). Furthermore, patients with 4G/4G genotype (r=0.28, p<0.001) had significantly higher plasma PAI-1 levels than those with 5G/5G genotype (r=0.02, p=0.4511). In addition, in a multiple logistic regression model, adjusted for all the other variables, PAI-1 was observed to be independently associated with CAD > 70% (p<0.001).

Conclusion: The most important finding of this study was the association between 4G/4G genotype, high plasma PAI-1 levels and coronary stenosis higher than 70% in Brazilian individuals. Whether high plasma PAI-1 levels are a decisive factor for atherosclerosis worsening or it is a consequence remains to be established. (Arq Bras Cardiol. 2011; [online]. ahead print, PP.0-0)

Keywords: Plasminogen activator inhibitor 1/genetics; polymorphism, genetic; coronary disease.
Methods

Individuals and study design

The approval for the study was granted by the Hospital Socor and Ethical Research Committee of the Universidade Federal de Minas Gerais. Informed consent was obtained from all participants, and the relevant clinical details for each individual were recorded. These details included personal and demographic data, family history, risk factors and coronary angiographic results that were initially recorded by three independent cardiologists (Table 1). A total of 123 male and female individuals attending the coronary angiography clinic in the Hemodynamic Department of Hospital Socor of Belo Horizonte, Minas Gerais, Brazil, with ages ranging from 42 to 69 years, were studied. Based on coronary angiography results, the participants were classified into three groups: angiographically normal arteries (n = 35), mild/moderate atheromatosis (n = 31) and severe atheromatosis (n = 57). The severe atheromatosis group was also subdivided according to the number of affected arteries into severe atheromatosis in one vessel (SA-1, n = 16), two vessels (SA-2, n = 13) and three or more affected blood vessels (SA-3, n = 28). All patients had a history of stable angina. However, none had a recent myocardial infarction or unstable angina (at least within the last three months), congestive heart failure, coagulation disorders, renal problems, hepatic or autoimmune diseases or cancer or were on warfarin treatment (at least within the last three months), congestive heart failure, coagulation disorders, renal problems, hepatic or autoimmune diseases or cancer or were on warfarin treatment. Further, none had a recent myocardial infarction or unstable angina. The mean age and body mass index (BMI) similar to the average of three groups of patients. However, this study was not intended to develop a case-control study type.

Angiography

Coronary angiography was performed via percutaneous transfemoral approach. The images were digitally recorded and all angiograms were analyzed by three experienced cardiologists who were not aware of the patients’ clinical details. Only patients who had three identical awards were selected. The extent and the progression of angiographically documented coronary arteries were classified as follows:

1. Normal coronary arteries (No angiographic stenosis)
2. Mild (Up to 30% stenosis of the luminal diameter in one or more coronary arteries)
3. Moderate (30 to 70% stenosis of the luminal diameter in one or more coronary arteries)
4. Severe (Greater than 70% stenosis of the luminal diameter in one or more coronary arteries)

Sample collection and laboratory determinations

Fasting peripheral venous blood samples were collected from each participant prior to angiography. Four and a half mL of blood was collected into vacutainer tubes containing 3.8% trisodium citrate anticoagulant (Becton-Dickinson) using a 21-gauge needle. Additionally, 5mL was collected in a tube without anticoagulant and 5mL with EDTA. Samples were immediately centrifuged for 15 minutes at 1100g. Plasma and serum were immediately isolated and aliquots were stored at -70°C for batch-wise analysis. The EDTA blood samples were submitted to genomic DNA extraction using the Wizard purification system (Promega, Inc.). The PAI-1 dosage was performed using plasma aliquots with the IMUBIND® Plasma PAI-1 ELISA (American Diagnostica® Inc. – USA) kit in accordance to instructions from manufacturer.

The 4G/5G polymorphism was determined using allele-specific polymerase chain reaction (PCR), as previously described®. PCR reactions were performed in a PT100 PCR thermocycler (MJ Research, Waltham, USA) using 1 pMol of each primer (Invitrogen®, São Paulo, SP), 0.2 mM of dNTPs (GIBCO BRL®, São Paulo, SP), and 1 unit of Taq polymerase (Phoneutria®-Belo Horizonte, Minas Gerais, Brazil). PCR reactions were submitted to 15 cycles consisting of 1 min at 95°C for denaturing, 1 min at 59°C for primer annealing and 1 min at 72°C for primer extension, preceded by an initial denaturing at 95°C for 5 minutes, followed by 20 cycles with girdling at 54°C and finishing with a pitch of 72°C for 7 minutes. DNAs from previously typed individuals were included in each sample set in order to control enzyme activity. Samples were then analyzed by silver stained acrylamide gel electrophoresis.
Results

The characterization of groups, including sex and age, as well as the risk factors associated with atherosclerosis, number of individuals and percentage of each variable are shown in Table 1. The four groups studied presented homogeneity with respect to age, sex and body mass index. A significant difference was observed for the smoking parameter for the severe atheromatosis group compared to the angiographically normal group (p = 0.02). Regarding a sedentary lifestyle, mild/moderate and severe atheromatosis groups were significantly different from the control and angiographically normal group (p < 0.05). Conventional lipid profile parameters are also presented in Table 1 as the means and standard deviations. No significant differences were found between groups for total cholesterol, HDL and LDL. Only triglyceride levels were significantly lower in the control group compared to the others. The results for plasma PAI-1 and 4G/5G polymorphism are shown in Table 2. The plasma PAI-1 levels were significantly higher in the severe atheromatosis group than in the others (p<0.001). The severe atheromatosis group also appear to have higher frequency of 4G/4G genotype and lower frequency of 4G/5G compared to the other groups. No significant difference was seen in the 5G/5G genotype between groups. No deviation from the Hardy-Weinberg equilibrium was observed.

When the severe atheromatosis group was subdivided, participants with one affected blood vessel presented higher PAI-1 levels compared with two and three affected blood vessels (Figure 1).

A linear regression showed that PAI-1 can be predicted from a linear combination of CAD (r = 0.47; p < 0.0001). Indeed, since these results were analyzed by using a binary logistic regression model, adjusted for all the other variables, PAI-1 seems to be independently associated with CAD > 70% (p < 0.001). This observation was confirmed by the odds ratio obtained for PAI-1 (2.358; 95%; CI = 1.085 – 9.432; p < 0.001). In addition, plasma PAI-1 levels showed a positive and significant association with 4G/4G genotype (r = 0.28, p < 0.001).

Discussion

Several authors have demonstrated the association of high PAI-1 levels with the presence of CAD. These results support the concept of the contribution of fibrin in intravascular atherothrombogenesis⁹. Decreased fibrinolytic activity, mainly caused by the increase in plasma PAI-1 concentration, has been associated with CAD in several studies¹⁰. In this study, plasma PAI-1 levels were significantly higher in the severe atheromatosis group compared to the other groups (Table 2). The averages obtained for SA-1, SA-2, and SA-3 subgroups were also significantly high compared to the control and angiographically normal group (Figure 1). These data show an association between the plasma PAI-1 concentration and CAD severity, considering the percentage of stenosis of more than 70%, regardless of the number of affected arteries. After adjusting for risk factors and other variables, the results of the multivariate analysis be inferred about an independent association between plasma PAI-1 levels and the presence of coronary artery stenosis greater than 70% (p < 0.001). The SA-1 subgroup also showed significant difference compared to the mild/moderate atheromatosis group (p = 0.004, Figure 1).

Another finding of this study was the association between 4G/4G genotype, increased plasma PAI-1 levels (r = 0.28, p < 0.001) and coronary artery stenosis with obstruction greater than 70%, suggesting that the presence of 4G allele may have influenced the increase in plasma PAI-1 levels, and that this increase may have affected the fibrinolytic system.

Table 1 – Characterization of the groups studied

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Angiographically Normal Arteries</th>
<th>Mild/Moderate Atheromatosis</th>
<th>Severe Atheromatosis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>38</td>
<td>35</td>
<td>31</td>
<td>57</td>
<td>-----</td>
</tr>
<tr>
<td>Men</td>
<td>18 (47%)</td>
<td>16 (46%)</td>
<td>17 (55%)</td>
<td>31 (54%)</td>
<td>0.341</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 ± 6</td>
<td>59 ± 7</td>
<td>59 ± 9</td>
<td>60 ± 8</td>
<td>0.299</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>26 ± 4</td>
<td>25 ± 4</td>
<td>26 ± 5</td>
<td>25 ± 3</td>
<td>0.204</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>0</td>
<td>6 (17%)</td>
<td>8 (25%)</td>
<td>23 (40%)</td>
<td>0.020</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>31 (88%)</td>
<td>25 (80%)</td>
<td>48 (84%)</td>
<td>0.543</td>
</tr>
<tr>
<td>Sedentary Lifestyle</td>
<td>20 (52%)</td>
<td>33 (94%)</td>
<td>23 (74%)</td>
<td>43 (75%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Family History</td>
<td>9 (23%)</td>
<td>14 (40%)</td>
<td>18 (58%)</td>
<td>29 (50%)</td>
<td>0.124</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>5 (14%)</td>
<td>7 (22%)</td>
<td>8 (14%)</td>
<td>0.354</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>179 ± 40</td>
<td>206 ± 49</td>
<td>203 ± 56</td>
<td>201 ± 44</td>
<td>0.821</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47 ± 8</td>
<td>43 ± 9</td>
<td>43 ± 9</td>
<td>42 ± 9</td>
<td>0.734</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>113 ± 36</td>
<td>129 ± 45</td>
<td>125 ± 46</td>
<td>127 ± 39</td>
<td>0.811</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>96 ± 43</td>
<td>167 ± 73</td>
<td>170 ± 82</td>
<td>159 ± 66</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significant differences between the groups are indicated by the symbol: * vs controls (ANOVA after log-transformation).
system, being thus a decisive factor for the progression of atherosclerosis in the individuals evaluated. Previous studies had demonstrated the association between 4G allele, high PAI-1 concentrations and CAD\textsuperscript{12-16}. Although this may be a promising correlation, it may not be demonstrated in all populations\textsuperscript{17-19}. This is the first study to demonstrate this correlation in the Brazilian population. Plasma PAI-1 levels may be influenced by several factors\textsuperscript{20}, among which genetic factors. Hong et al\textsuperscript{21} showed that 42% of the variations found in plasma PAI-1 levels were due to genetic factors, in a study involving the Swedish population. Naram et al\textsuperscript{11}, examining the South African population, observed that lower plasma PAI-1 levels were obtained for the black population compared with Caucasians. The authors

Table 2 – Plasma PAI-1 levels according to PAI-1 genotype

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Angiographically Normal Arteries</th>
<th>Mild/Moderate Atheromatosis</th>
<th>Severe Atheromatosis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>38</td>
<td>35</td>
<td>31</td>
<td>57</td>
<td>----</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>28.1 (22.9 – 38.7)</td>
<td>34.9 (27.9 – 42.4)</td>
<td>40.5 (34.2 – 51.8)</td>
<td>66.6 (44.7 – 91.6) \textsuperscript{*}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4G/4G</td>
<td>12 (31%)</td>
<td>10 (28%)</td>
<td>9 (29%)</td>
<td>27 (43%) \textsuperscript{†}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>27.9 (23.6 – 38.4)</td>
<td>31.0 (25.4 – 36.2)</td>
<td>33.8 (31.8 – 50.9)</td>
<td>45.8 (28.4 – 74.6) \textsuperscript{*}</td>
<td>0.002</td>
</tr>
<tr>
<td>5G/5G</td>
<td>14 (36%)</td>
<td>12 (34%)</td>
<td>11 (35%)</td>
<td>20 (36%)</td>
<td>0.434</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>32.0 (19.9 – 39.1)</td>
<td>38.3 (23.4 – 52.6)</td>
<td>49.9 (39.1 – 55.2)</td>
<td>74.4 (47.7 – 95.6) \textsuperscript{*}</td>
<td>0.005</td>
</tr>
<tr>
<td>4G/5G</td>
<td>12 (31%)</td>
<td>13 (37%)</td>
<td>11 (35%)</td>
<td>10 (22%) \textsuperscript{†}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>27.2 (23.8 – 37.6)</td>
<td>34.9 (31.1 – 39.1)</td>
<td>39.6 (36.9 – 46.9)</td>
<td>84.1 (64.1 – 102.5) \textsuperscript{*}</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PAI-1 levels are expressed as median (interquartile range) and percentage presence of the 4G/5G polymorphism in the groups studied. \textsuperscript{*} and \textsuperscript{†} indicates significant difference vs all the other groups, \textsuperscript{*} Kruskal-Wallis, \textsuperscript{†} Chi-square test.

Figure 1 – Distribution of plasma PAI-1 levels for controls, angiographically normal arteries (NAn), mild/moderate atheromatosis (MMA) and severe atheromatosis (SA) groups with one (SA-1), two (SA-2) and three or more affected vessels (SA-3). Each box represents the median and interquartile range of values, with the I bars (whiskers) extended to the minimum and maximum values. The gray area represents the reference values (0 - 47ng/mL). \textsuperscript{6} Indicates significant differences compared to controls, NAn and MMA groups (p<0.01).
concluded that the main reason for the detection of lower PAI-1 levels in black individuals was the low prevalence of 4G allele in this group of individuals. Although ethnicity influences plasma PAI-1 levels, this study showed no significant difference in PAI-1 levels for the different ethnic groups. The study design could justify such finding, because the group studied in this work (n=161) was composed of 30.9% of Caucasians, 26.8% of blacks and 42.3% of mestizos, but uniformly distributed in the three groups, showing no statistically significant differences between them for different ethnicities. The frequency of 4G and 5G alleles described for the Brazilian population, which presents a variety of ethnic groups, has not yet been fully established in literature. In this study, 51% of participants (n=161) had 4G allele, while 49% had 5G allele.

Whether an increase in plasma PAI-1 levels is a decisive factor for worsening of atherosclerosis or its increased levels are a consequence of this injury still to be clarified. PAI-1 has been studied in different settings with thrombotic pathophysiology. PAI-1 also plays an important role in signal transduction, cell adherence, and migration. In a diseased artery environment there is a heterogeneous population of cells, all of which may be synthesizing PAI-1. These observations suggest that PAI-1 may be involved in CAD as a result of the initialization and progression of atheromatosis with enhanced fibrin accumulation, either by a fibrinolysis impaired effect or by enhancing the feedback synthesis response.

### Conclusion

The most important finding of this study was the association between 4G/4G genotype, high plasma PAI-1 levels and coronary stenosis higher than 70% in Brazilian individuals. The relationship observed with severe atheromatosis was irrespective of smoking, arterial hypertension, sedentary lifestyle, family history and lipid profile. These data support the hypothesis that PAI-1 plays a role in the pathogenesis of atherosclerosis or its major clinical manifestation, CAD. Whether high plasma PAI-1 levels are a decisive factor for atherosclerosis worsening or it is a consequence of it, remains to be established.

### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

### Sources of Funding

This study was partially funded by CNPq, CAPES and FAPEMIG.

### Study Association

This article is part of the thesis of doctoral submitted by Luciana Moreira Lima, from Universidade Federal de Minas Gerais.

### References


### Arq Bras Cardiol. 2011; [online].ahead print, PP.0-0


