INTRODUCTION

Coronary artery disease (CAD) is the single most common cause of death in the developed countries of Europe and United States and also in developing countries.1-3 Atherosclerosis of one or more major branches of the coronary arteries is the underlying abnormality in ischemic heart disease (IHD).4,5 Acute disorders like acute myocardial infarction, unstable angina and sudden cardiac death develop with more than 50% stenosis of coronary arteries.1,6

Pregnancy-associated plasma protein-A (PAPP-A) is a zinc binding metalloproteinase belonging to metzincin superfamily.7,8 It circulates either as an active homodimer (free form) or covalently bound to the proform of eosinophil major basic protein (ProMBP).7 Circulating acute coronary syndrome (ACS)-related PAPP-A is different from circulating pregnancy-related PAPP-A in not being complexed with ProMBP. The molecular size of pregnancy-related PAPP-A is also larger than that of ACS-related PAPP-A.8 PAPP-A is used as a serum marker for prenatal diagnosis of Down syndrome during pregnancy. Only recently, its utility has been found other than pregnancy as well.7-9 PAPP-A is expressed in a variety of tissues including ovary, endometrium, testes, kidney, and colon and is also produced by human and porcine coronary artery vascular smooth muscle cells.10,11

PAPP-A functions as a protease. It regulates free IGF-I activity which is involved in vascular smooth muscle growth and extracellular matrix synthesis in the atherosclerotic plaque formation.12,13 IGF-I also promotes macrophage activation, chemotaxis, low density lipoprotein (LDL) cholesterol uptake by macrophages, and the release of proinflammatory cytokines, which are known to play a role in the development of atherosclerotic lesions.14-16

Elevated levels of PAPP-A in serum has been advocated as a marker of ACS.16-18 In cardiac troponin-I (cTnI) negative ACS patients, PAPP-A levels greater than 2.9 mIU/L were associated with a higher risk of adverse outcome compared with patients whose circulating PAPP-A levels were < 2.9 mIU/L.11 As PAPP-A regulates free IGF-I activity so it also promotes IGF-I dependent actions on vasculogenesis, vasodilation, cell preconditioning and cell survival. This explains PAPP-A

ABSTRACT

Objective: To compare pregnancy-associated plasma protein-A (PAPP-A) levels in individuals with and without coronary artery disease (CAD).

Study Design: Cross-sectional comparative study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, in collaboration with Armed Forces Institute of Cardiology (AFIC), from September 2008 to March 2010.

Methodology: One hundred and twenty five (125) individuals both male and female were included in the study. Blood for PAPP-A and lipid profile was collected, just before angiography. On the basis of angiography, the individuals were divided into those with and without CAD. PAPP-A was analyzed by using Diagnostic System Laboratories (DSL) Enzyme Linked Immunosorbet Assay (ELISA) kit and reading was taken by ELISA reader. Lipid profile was determined on automated analyzers Selectra-2 and Vitros 5.1.

Results: Amongst the 125 individuals, 41 individuals were without CAD whereas 84 individuals were having CAD. Mean PAPP-A levels were 0.74 ± 0.35 mIU/L in those without CAD whereas mean PAPP-A levels in those with CAD were 1.35 ± 0.57 mIU/L. The difference between the two groups was statistically significant (p < 0.001). A PAPP-A cut off level of 0.85 mIU/L had a sensitivity and specificity of 78% and 70% respectively for diagnosing atherosclerotic CAD.

Conclusion: PAPP-A is a potentially relevant marker of the presence and extent of coronary atherosclerosis as its levels are elevated in CAD as compared to individuals without CAD.


ORIGINAL ARTICLE

Pregnancy-Associated Plasma Protein-A Levels in Individuals with and without Coronary Artery Disease

Najeeb Ullah Khan, Farooq Ahmad Khan, Dilshad Ahmed Khan and Nowshad Asim

INTRODUCTION

Coronary artery disease (CAD) is the single most common cause of death in the developed countries of Europe and United States and also in developing countries.1-3 Atherosclerosis of one or more major branches of the coronary arteries is the underlying abnormality in ischemic heart disease (IHD).4,5 Acute disorders like acute myocardial infarction, unstable angina and sudden cardiac death develop with more than 50% stenosis of coronary arteries.1,6

Pregnancy-associated plasma protein-A (PAPP-A) is a zinc binding metalloproteinase belonging to metzincin superfamily.7,8 It circulates either as an active homodimer (free form) or covalently bound to the proform of eosinophil major basic protein (ProMBP).7 Circulating acute coronary syndrome (ACS)-related PAPP-A is different from circulating pregnancy-related PAPP-A in not being complexed with ProMBP. The molecular size of pregnancy-related PAPP-A is also larger than that of ACS-related PAPP-A.8 PAPP-A is used as a serum marker for prenatal diagnosis of Down syndrome during pregnancy. Only recently, its utility has been found other than pregnancy as well.7-9 PAPP-A is expressed in a variety of tissues including ovary, endometrium, testes, kidney, and colon and is also produced by human and porcine coronary artery vascular smooth muscle cells.10,11

PAPP-A functions as a protease. It regulates free IGF-I activity which is involved in vascular smooth muscle growth and extracellular matrix synthesis in the atherosclerotic plaque formation.12,13 IGF-I also promotes macrophage activation, chemotaxis, low density lipoprotein (LDL) cholesterol uptake by macrophages, and the release of proinflammatory cytokines, which are known to play a role in the development of atherosclerotic lesions.14-16

Elevated levels of PAPP-A in serum has been advocated as a marker of ACS.16-18 In cardiac troponin-I (cTnI) negative ACS patients, PAPP-A levels greater than 2.9 mIU/L were associated with a higher risk of adverse outcome compared with patients whose circulating PAPP-A levels were < 2.9 mIU/L.11 As PAPP-A regulates free IGF-I activity so it also promotes IGF-I dependent actions on vasculogenesis, vasodilation, cell preconditioning and cell survival. This explains PAPP-

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production in damaged tissues as a promoter of repair.9-11 Even mild damage, such as brief ischemia, would activate this pathway, thus explaining the higher sensitivity of PAPP-A compared with cardiac troponins as predictor of outcome.11 The PAPP-A threshold level of 4.5 mIU/L has the highest combined sensitivity (45%) and specificity (84%) for the identification of CAD, thus PAPP-A may be both a marker of atherosclerotic CAD and a pathogenic agent in coronary atherosclerosis.16,19 PAPP-A levels have been found in patients with stable angina to correlate with more complex stenosis and the CAD extent on angiography.7,18 Chronic stable angina (CSA) patients with multi-vessel disease had significantly higher PAPP-A levels than those with single vessel disease and patients without CAD.18 PAPP-A is emerging as a new marker for CAD as well as having correlation with degree of coronary atherosclerosis. The purpose of this study was to determine the levels of PAPP-A in CAD and to establish the association of PAPP-A with atherosclerosis in coronary arteries.

METHODOLOGY

This cross-sectional comparative study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi in collaboration with Armed Forces Institute of Cardiology (AFIC) and National Institute of Heart Disease (NIHD), Rawalpindi.

The study was conducted after approval by the institutional Ethical Committee. It was conducted from September 2008 to March 2010. A total of 125 individuals coming to AFIC and NIHD, Rawalpindi for angiography, irrespective of age and gender, were included in the study. After reassurance and consent, angiography was performed by Judkin technique using a quantitative coronary angiography system.20 Individuals with more than 50% stenosis of coronary arteries were labelled as patients of CAD and those with less than 50% stenosis were labelled as individuals without CAD. The extent of CAD was based on the number according to the stenosis in vessels i.e single, double and triple vessel disease. Individuals with advanced kidney or liver failure, overt heart failure, history of major surgery or trauma within the previous two months, with known or systemic thrombotic disorder (other than those of coronary origin), inflammatory disease and pregnant women were excluded.

Sample collection was performed between 0600-0700 hours. Five ml venous blood sample for lipid profile and PAPP-A was drawn using aseptic technique. Angiography of the individuals was then performed. The tubes were properly labelled and the specimens were transported to the processing room within half an hour and allowed to clot at room temperature. Serum was then separated by centrifugation at a relative centrifugal force of 2000-3000 G for about 15 minutes.

The lipid profile was done on the same day using standard protocols. Total cholesterol and triglycerides analysis was done on random access automated analyzer Selectra-2 by Merck and HDL cholesterol was analyzed on random access automated analyzer Vitros 5.1 by Jhonson. LDL cholesterol was calculated by Friedwald formula. The serum specimens for PAPP-A were stored at -20°C and analysis was done in batches. PAPP-A was measured by DSL-10-27100 ACTIVE PAPP-A ELISA, an enzymatically amplified “two-step” sandwich-type immunoassay.21 PAPP-A antibodies were reacted with standards, controls and sample. Then absorbance was measured for each analyte. This absorbance measured was directly proportional to the concentration of PAPP-A present.

All the data was compiled for statistical analysis using Statistical Package for Social Sciences Programme (SPSS) Version 16.0. Descriptive statistics were carried out to summarize the data. Mean and standard deviation were calculated for quantitative variables like age, lipid profile and PAPP-A levels in patients of CAD and without CAD. Frequencies and percentages were calculated for qualitative variables like gender, smoking status, diabetes and hypertension.

Independent sample t-test was used to compare PAPP-A levels and lipid profile between individuals with CAD and without CAD. Chi-square test was used to compare gender, smoking status, hypertension status, diabetes and dyslipidemia status between the two groups. At 5% level of significance, p values less than 0.05 were considered significant. Receiver Operating Characteristics (ROC) curve was used to establish threshold value for PAPP-A that would be used for the diagnosis of CAD.

RESULTS

Mean age of the individuals with CAD was 57.6 ± 8.3 years. The mean age of the individuals without CAD was 48.9 ± 9.2 years (Table I). The difference in age between the two groups was statistically significant (p < 0.001). Angiographically 41 out of 125 (32.8%) individuals did not have CAD whereas 84 individuals had CAD. Of the 84 individuals with CAD, 75 were males, while 9 were females. Twenty two (17.6%) had single vessel CAD, 32 (25.6%) had double vessel CAD and 30 (24%) had triple vessel CAD. Other baseline characteristics of the individuals are given in Table I.

Mean PAPP-A levels were 0.74 ± 0.35 mIU/L in those without CAD, whereas mean PAPP-A levels in those with CAD were 1.35 ± 0.57 mIU/L. Difference between the two groups was statistically significant as depicted by p-value of < 0.001. PAPP-A levels in single vessel
CAD were 0.98 ± 0.33 mIU/L in double vessel CAD were 1.33 ± 0.50 mIU/L and in triple vessel CAD were 1.64 ± 0.61 mIU/L. Lipid profile of the individuals is shown in Table II.

ROC curve was drawn to know the sensitivity and specificity of PAPP-A for diagnosing CAD (Figure I). Area under the curve was 0.825, standard error was 0.037 and asymptomatic significance was < 0.001. A PAPP-A cutoff level of 0.85 mIU/L had a sensitivity and specificity of 78% and 70% respectively for diagnosing atherosclerotic CAD.

Table I: Baseline characteristics of individuals with and without CAD (n=125).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without CAD</th>
<th>CAD patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 41</td>
<td>n = 84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.9 ± 9.2</td>
<td>57.6 ± 8.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>30 (73%)</td>
<td>75 (89.2%)</td>
<td>0.0210</td>
</tr>
<tr>
<td>Female (%)</td>
<td>11 (27%)</td>
<td>9 (10.8%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>9 (22%)</td>
<td>46 (55%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypertensives taking medications (%)</td>
<td>15 (37%)</td>
<td>57 (68%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetics (%)</td>
<td>10 (25%)</td>
<td>37 (44%)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Dyslipidemias (%)</td>
<td>9 (22%)</td>
<td>36 (43%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table II: Mean values of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and PAPP-A levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without CAD Mean ± SD</th>
<th>CAD Patients Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP-A levels (mIU/L)</td>
<td>0.74 ± 0.35</td>
<td>1.35 ± 0.57</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol levels (mmol/L)</td>
<td>4.30 ± 0.78</td>
<td>4.45 ± 1.10</td>
<td>0.436</td>
</tr>
<tr>
<td>Triglycerides levels (mmol/L)</td>
<td>1.65 ± 0.72</td>
<td>2.01 ± 0.68</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL cholesterol levels (mmol/L)</td>
<td>1.02 ± 0.26</td>
<td>0.90 ± 0.14</td>
<td>0.0010</td>
</tr>
<tr>
<td>LDL cholesterol levels (mmol/L)</td>
<td>2.50 ± 0.80</td>
<td>2.57 ± 0.86</td>
<td>0.663</td>
</tr>
</tbody>
</table>

The present results showed that mean PAPP-A levels were significantly lower in those without CAD than in those with CAD. Meanwhile PAPP-A levels in single vessel CAD were lower than in double vessel CAD which, in turn was lower than in triple vessel CAD. This shows that PAPP-A levels were found to be higher in individuals with multivessel disease than with single vessel disease. Although these levels were lower than those reported by some of the studies on PAPP-A, due to difference in the type of kit used for PAPP-A estimation.7,9-11

These findings are consistent with the findings by Cosin-Sales et al., in which PAPP-A levels in patients with stable angina were found to correlate with more complex stenosis and the CAD extent on angiography. PAPP-A levels were found to be higher in patients with multi-vessel disease than in those with single-vessel disease.18 In a study by Mueller et al., the median PAPP-A levels in patients with peripheral atherosclerotic disease (PAD) and in patients without atherosclerotic disease were 0.81 vs 0.64 mU/L (p < 0.001) respectively.19 In a study by Onder et al. concentration of PAPP-A was significantly lower in CCB users than in nonusers.26 However, they also suggested that PAPP-A may not have a causal effect in the determination of coronary syndromes, but can rather represent a sensitive signal of damage, similar to those exerted by other acute phase proteins, being part of an endogenous compensatory pathway aimed at tissue physiological repair and replicative programs.26

In this study, serum total cholesterol, triglycerides, and LDL cholesterol were found to be higher while HDL cholesterol was lower in individuals with CAD. This is in consensus with the study by Aso et al. In patients with type 2 diabetes, serum concentrations of PAPP-A correlated positively with serum total cholesterol and LDL cholesterol.10 There is reported to be a positive correlation between carotid intimal thickness, which is an early marker of atherosclerosis and raised serum PAPP-A.10

DISCUSSION

Atherosclerotic CAD and its progression leading to ACS is rather an unpredictable phenomenon. The fact that both atherogenesis and atherosclerotic CAD progression are directly linked to inflammatory mechanisms has generated interest regarding the potential role of inflammatory molecules as markers of atherogenesis and cardiovascular risk. CRP, a marker of inflammation, has been shown to add value to the predictive ability of conventional risk factors. However, complex mechanisms are responsible for both disease progression and acute events. So it is unlikely that a single molecule can provide clinicians with an accurate prediction of cardiovascular risk. Currently utilized markers, including CRP, are non-specific. Research is going on for better markers of both atheromatous plaque activity and patient’s vulnerability. New markers of CAD progression have been identified in recent years, among which, PAPP-A appears to be a promising cardiac marker of atherosclerosis leading to CAD and adverse outcome.24,25

The lipid profile of the individuals is shown in Table II.
At a PAPP-A level of 0.85 mIU/L, it had a sensitivity and specificity of 78% and 70% respectively for diagnosing atherosclerotic CAD. So PAPP-A levels were able to correspond with the degree of atherosclerosis on angiography. Different cutoff values of PAPP-A have been used to diagnose CAD and ACS and to predict the risk of further damage. A concentration > 4.5 mIU/L was found to predict the presence of significant (> 50%) stenoses with a sensitivity of 45% and a specificity of 84%.18 Lund et al. demonstrated that in cardiac Troponin-I (cTnI) negative ACS patients, PAPP-A levels greater than 2.9 mIU/L were associated with a higher risk of adverse outcome compared with patients whose circulating PAPP-A levels were < 2.9 mIU/L.11 In a similar study, Heeschen et al. documented that in patients with ACS, elevated PAPP-A levels > 12.6 mIU/l indicated an increased risk (odds ratio = 2.44, p < 0.001).21 Thus based upon these findings, measurement of PAPP-A may become a clinically useful tool in stable patients with chest pain, both to diagnose, or exclude, and to gauge the extent of obstructive atherosclerotic coronary lesions.

Because PAPP-A levels are relatively stable and no specific sampling conditions are required for PAPP-A, this marker may indeed be suitable for routine clinical use. In the future, PAPP-A may represent an important tool for predicting the degree of atherosclerosis leading on to develop CAD and in the diagnostic and therapeutic stratification of patients with ACS without evidence of myocardial necrosis and predicting prognosis after an attack of ACS.

CONCLUSION

PAPP-A is a potentially clinically relevant marker of the presence and extent of coronary atherosclerosis and its levels were elevated in individuals CAD as compared to those without CAD.

REFERENCES


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