Introduction

Indian subcontinent is facing an epidemic of coronary artery disease (CAD). It is house to nearly 70 million patients suffering from atherosclerotic cardiovascular disorders.1 Acute coronary syndromes – Unstable angina, non ST elevation myocardial infarction (NSTEMI) and ST elevation myocardial infarction (STEMI) are major causes of death in patients with CAD.2 Unstable atherosclerotic plaque is a major mechanism of development of acute coronary syndrome (ACS) from stable CAD.3 Several biomarkers of ACS have been identified. These include creatinine phosphokinase-MB (CPK-MB), cardiac troponin T (cTnT), brain natriuretic peptide, high sensitive C-reactive protein (hs-CRP), myeloperoxidase, pregnancy associated plasma protein-A (PAPP-A), metalloproteinase-9 etc.5 These biomarkers play important role in risk stratification and treatment strategies in patients with ACS.5,6 The availability of a sensitive and specific early marker of plaque instability, whose levels become elevated before or even in the absence of myocardial necrosis, should improve diagnostic and therapeutic decision making. Pregnancy Associated Plasma Protein-A is one such marker and its serum levels have been shown to rise further with the severity of ACS.7 Thus there is room for improvement regarding the available early diagnostic and risk assessment approaches used in the management of coronary patients.3,22 PAPP-A is a screening marker for Down’s syndrome pregnancies.7 It is also present in human fibroblasts and released during rupture of atherosclerotic plaque.8 Since PAPP-A was found to be elevated in patients of ACS in 2001, its role as either a diagnostic or prognostic marker for CAD has been an interesting area of research.7 The hypothesis behind current study is that the process of transformation from stable to unstable plaque might be reflected by increase in levels of PAPP-A.8 Unlike Troponin T or I, PAPP-A has fewer causes of false positivity, so it can be used for the accurate diagnosis of ACS.

Methods

Subjects and design

This study used an analytic cross-sectional design. Written informed consent was taken from both patients and controls prior to enrollment into the study. The study comprised of the following four groups with 30 males in each group between age 40-60 years. Group A: Healthy controls. Group B: Old myocardial infarction, not having features of acute coronary syndrome. Group C: unstable angina and non ST elevation myocardial infarction. Group D: ST elevation myocardial infarction (STEMI). Patients with renal disease [blood urea > 40 mg/dl, serum creatinine levels >1.5mg/dl] or post real transplant patients, inflammatory conditions on clinical history and examination, terminal

Abstract

Objectives: The availability of a sensitive and specific early marker of plaque instability, whose levels become elevated before or even in the absence of myocardial necrosis, should improve diagnostic and therapeutic decision making in Acute Coronary Syndromes. This analytic cross-sectional study was designed to estimate the serum levels of Pregnancy Associated Plasma Protein A (PAPP-A), highly sensitive C reactive protein (hs-CRP) and creatinine phosphokinase MB (CPK-MB) in patients of old myocardial infarction, unstable angina, non ST elevation and ST elevation myocardial infarction and to assess their correlation with plaque instability.

Methods: Male patients of Coronary Artery Disease aged between 40 to 60 years were recruited in to the four study groups: Healthy controls; Old Myocardial Infarction, not having features of acute coronary syndrome; Unstable Angina, non ST elevation myocardial infarction and ST elevation Myocardial Infarction. Appropriately timed blood sample collection was done and serum levels of PAPP-A, hs-CRP and CPK-MB were estimated. Qualitative cardiac troponin T was done in all patients. Appropriate statistical tests were applied and intergroup comparison was done.

Results: Serum levels of PAPP-A were found to be significantly different in all the four groups (p<0.001) with highest values observed in patients of ST elevation myocardial infarction (26.38 ± 4.10 IU/l) as compared to controls (3.29 ± 0.93). The serum levels of PAPP-A has a statistically significant positive correlation with the mean serum levels of CPK-MB with a correlation coefficient (R²) of 0.781 and a p value of <0.001. Thus, it may be useful in diagnosing ACS, especially in cardiac troponin T negative patients.

Conclusion: Serum levels of PAPP-A is a sensitive and specific early marker of plaque instability, whose levels become elevated before or even in the absence of myocardial necrosis. This marker can improve diagnostic and therapeutic decision making in Acute Coronary Syndromes.

Relationship between Serum Levels of Pregnancy Associated Plasma Protein A and Coronary Artery Disease in Males

Prattay Guha Sarkar1*, Gabjender Ranga2

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Table 1: Comparison of serum hs-CRP levels in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (mg/dl)</th>
<th>Interquartile Range (mg/dl)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Control) (n=30)</td>
<td>2.44 ± 0.63</td>
<td>1.97-2.95</td>
<td>vs B &lt; 0.001, vs C &lt; 0.001, vs D &lt; 0.001, A vs D = 0.01</td>
</tr>
<tr>
<td>Group B (Old MI) (n=30)</td>
<td>4.20 ± 0.98</td>
<td>3.67-5.10</td>
<td></td>
</tr>
<tr>
<td>Group C (UA/NSTEMI) (n=30)</td>
<td>5.52 ± 1.81</td>
<td>3.77-7.12</td>
<td></td>
</tr>
<tr>
<td>Group D (STEMI) (n=30)</td>
<td>8.80 ± 1.60</td>
<td>7.37-9.62</td>
<td></td>
</tr>
</tbody>
</table>

p values significant at <0.05 and highly significant at <0.001; All values have been described as mean ± SD and range

Table 2: Comparison of serum CPK-MB in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (IU/dl)</th>
<th>Interquartile range (IU/dl)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Control) (n=30)</td>
<td>18.27 ± 4.04</td>
<td>15-21</td>
<td>vs B &lt; 0.001, vs C &lt; 0.001, vs D &lt; 0.001, A vs B = 0.01</td>
</tr>
<tr>
<td>Group B (Old MI) (n=30)</td>
<td>18.93 ± 3.67</td>
<td>16.75-21.25</td>
<td></td>
</tr>
<tr>
<td>Group C (UA/NSTEMI) (n=30)</td>
<td>77.67 ± 75.85</td>
<td>21-154.50</td>
<td></td>
</tr>
<tr>
<td>Group D (STEMI) (n=30)</td>
<td>396.57 ± 163.70</td>
<td>257.25-458.50</td>
<td></td>
</tr>
</tbody>
</table>

p values significant at <0.05 and highly significant at <0.001; All values have been described as mean ± SD and range

Table 3: Comparison of serum levels of PAPP-A in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (mIU/l)</th>
<th>Interquartile Range (mIU/l)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Control) (n=30)</td>
<td>3.29 ± 0.93</td>
<td>2.22-4.01</td>
<td>vs B &lt; 0.001, vs C &lt; 0.001, vs D &lt; 0.001, A vs B = 0.01</td>
</tr>
<tr>
<td>Group B (Old MI) (n=30)</td>
<td>8.66 ± 1.13</td>
<td>7.86-9.64</td>
<td></td>
</tr>
<tr>
<td>Group C (UA/NSTEMI) (n=30)</td>
<td>16.02 ± 4.11</td>
<td>12.38-19.82</td>
<td></td>
</tr>
<tr>
<td>Group D (STEMI) (n=30)</td>
<td>26.38 ± 4.10</td>
<td>23.65-29.68</td>
<td></td>
</tr>
</tbody>
</table>

p values significant at <0.05 and highly significant at <0.001.; All values have been described as mean ± SD and range

Table 4: Correlation of serum levels of PAPP-A with hs-CRP and CPK-MB in study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAPP-A (n=120)</th>
<th>CPK-MB (n=120)</th>
<th>hs-CRP (n=120)</th>
<th>p value PAPP-A vs CPK-MB</th>
<th>p value PAPP-A vs hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.781</td>
<td>0.840</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

p-value significant at <0.05 and highly significant at <0.001

Illness like terminally ill patients of cancer, patients on ventilator, multi organ dysfunction and patients with immunocompromising conditions like cancer chemotherapy, corticosteroid therapy, HIV infections were excluded from the study. Routine Investigations, ECG, qualitative Troponin T level estimated using commercially available kit, Serum CPK-MB, Serum hs-CRP levels and Serum PAPP-A were done on all recruited patients and healthy controls.

Statistical Analysis

One way ANOVA was used to calculate p-values for separate groups of parametric data. Kruskal-Wallis and Wilcoxon W test was used to calculate p-value for separate groups of non-parametric data. Spearman’s rank order correlation was used to determine coefficients for nonparametric measures of association. A p-value < 0.05 was considered to be statistically significant and a p-value < 0.001 was considered to be highly significant.

Results

Serum level of hs-CRP was found to increase with the severity of coronary artery disease. Mean value of hs-CRP in group A (controls), B (old myocardial infarction), C (UA/NSTEMI) and D (STEMI) were found to be 2.44 ± 0.63 mg/dl, 4.20 ± 0.98 mg/dl, 5.52 ± 1.81 mg/dl and 8.80 ± 1.60 mg/dl respectively. There was statistically significant difference in the serum level of hs-CRP in all the four groups (p < 0.05) (Table 1).

Serum level of CPK-MB was also found to increase with the escalating severity of coronary artery disease. It was normal in controls, patients of old myocardial infarction and patients of unstable angina. Mean values of CPK-MB in group A (controls), B (old myocardial infarction), C (UA/NSTEMI) and D (STEMI) were found to be 18.27 ± 4.04, 18.93 ± 3.67, 77.67 ± 75.85 and 396.57 ± 163.70 IU/l respectively. There was statistically significant difference in the serum level of CPK-MB in all the four groups (p < 0.001) (Table 2).

Serum levels of PAPP-A were found to be significantly different in all the four groups. Highest values were observed in patients of ST elevation myocardial infarction. Mean values of PAPP-A in group A (controls), B (old myocardial infarction), C (UA/NSTEMI) and D (STEMI) were found to be 3.29 ± 0.93, 8.66 ± 1.13, 16.02 ± 4.11 and 26.38 ± 4.10 IU/l respectively (Table 3).

The serum levels of PAPP-A had a statistically significant positive correlation with the mean serum levels of CPK-MB with a correlation coefficient (R²) of 0.781 and a p value of <0.001. Furthermore, the serum levels of PAPP-A also has a statistically significant positive correlation with the serum levels of hs-CRP with a correlation coefficient (R²) of 0.840 and a p value of <0.001. (Table 4).

Intergroup analysis of correlation between CPK-MB and PAPP-A revealed that serum levels of PAPP-A correlated strongly with the serum levels of CPK-MB in patients of UA/NSTEMI and STEMI. Correlation coefficient (R²) calculated in patients of UA/NSTEMI is 0.777 and in patients of STEMI is 0.812. However, the correlation of serum levels of PAPP-A with CPK-MB was not significant statistically in patients of old myocardial infarction and healthy controls. Mean serum levels of PAPP-A correlated weakly with the mean serum levels of hs-CRP in patients of UA/NSTEMI and patients of STEMI.
p-value significant at <0.05 and highly significant at <0.001 (Table 5).

Further, mean serum levels of PAPP-A were found to correlate directly with the size of infarct in patients with STEMI. It was found to be highest in patients with anteroseptal STEMI compared to patients of inferior wall STEMI and anterior wall STEMI. Serum PAPP-A levels had a very strong correlation with mean serum CPK-MB levels in all patients of STEMI. Mean serum levels of PAPP-A were found to be highest in NYHA class IV patients compared to patients in other functional classes. Further, mean serum level of PAPP-A was higher in cTnT positive patients compared to cTnT negative patients and the difference was statistically significant.

**Discussion**

The results of our study show that all patient groups of CAD had a higher mean serum level of PAPP-A. Interestingly the levels increased progressively as the severity of CAD increased. On intergroup comparison it was found that the mean serum levels of PAPP-A were significantly different in all the four groups and the difference was highly significant statistically (p<0.001). It implies that serum levels of PAPP-A show a progressive rise with increase in unstable plaque burden in coronary artery disease. The results of our study are in accordance with the pioneering work done by Bayes-Genis et al. They had observed substantially and significantly higher PAPP-A levels in acute myocardial infarction and in unstable angina than in control subjects (20.6 mIU/L vs. 14.9 mIU/L vs. 7.4 mIU/L) with a p-value of <0.01.

Further, using a cut off value of 10 mIU/L it was possible to identify ACS patients with a sensitivity of 89.2% and specificity of 81.3%. The results of our study further highlight the value of PAPP-A as a robust biomarker for the diagnosis of ACS, as all our patients had values greater than 10 mIU/L. The lowest value in our patients of UA/NSTEMI was 11.23 mIU/L. A significantly lower serum level of PAPP-A was found in the control subjects of our study. If we use a cut off value of 10 mIU/L then the sensitivity of the test, according to our study, is 100%. It was found that the serum levels of PAPP-A were significantly higher in patients of STEMI as compared to other study subjects. Patients of UA and NSTEMI had serum levels of PAPP-A lower than patients of STEMI. Patients of old myocardial infarction with stable CAD had further lower serum levels of PAPP-A. Lowest serum levels of PAPP-A was found in the control subjects.

This progressive rise in the serum level of PAPP-A may be because of its release from unstable plaque and progressive increase in the plaque instability in patients of severe CAD and ACS. In view of the available data and the results of our study, it can be concluded that serum PAPP-A level is a highly sensitive and specific biomarker for ACS. The serum levels of PAPP-A correlate with the severity of ACS and can be used to predict the outcome of ACS.

A progressive increase in the serum levels of hs-CRP was noted with the increase in the severity of CAD. hs-CRP levels correlate with the clinical severity of coronary artery disease and with coronary events in both acute and sub-acute phase of myocardial ischemia. Patients who are hospitalized for treatment of acute coronary syndrome and have raised CRP levels have significantly more ischemic episodes during hospital stay than patients with lower CRP levels. Sub-group analysis also revealed that statistically significant correlation between the serum levels of PAPP-A and CPK-MB was present only in patients of UA/NSTEMI and STEMI. There was no statistically significant correlation found in healthy controls and patients of old myocardial infarction. Our results are in accordance with those of You et al. In stable CAD patients, CPK-MB levels do not increase, but as is evident from the results of our study, serum PAPP-A levels increase in the patients of stable CAD as compared to controls, reflecting the presence of potentially unstable plaque.

So it may be concluded from our study that PAPP-A in peripheral blood may reflect the unstability of plaque. It can help to accurately make diagnosis of ACS in Troponin negative patients or in patients with false positive Troponins. Future studies are required to assess further the serum levels of PAPP-A as a potential biomarker for plaque instability. It has to be seen whether it can predict plaque rupture in future. Larger studies with greater number of subjects are required to further confirm our observations.

**References**