Pregnancy Associated Plasma Protein-A (PAPP-A) Levels in Acute Coronary Syndrome: A Case Control Study in a Tertiary Care Centre

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Received: 25 September 2013 / Accepted: 15 January 2014 / Published online: 22 February 2014
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Abstract In recent years, an important objective of cardiovascular research has been to find new markers that would improve the risk stratification and diagnosis of patients presenting with symptoms of acute coronary syndrome (ACS). Established biomarkers for diagnosis of ACS includes troponins and creatine kinase MB (CK-MB). Pregnancy associated plasma protein A (PAPP-A) is an emerging marker which has been described as a marker of plaque instability. PAPP-A is a large metalloproteinase involved in insulin-like growth factor signaling and has been shown to be involved in pathological processes like atherosclerosis. Many studies have been published regarding release of PAPP-A in circulation during ACS. The objective of this study was to evaluate the role of PAPP-A as an early marker of ACS by comparing levels of PAPP-A in patients with acute myocardial infarction (AMI) and unstable angina (UA) with asymptomatic controls. The association of PAPP-A with markers of myocardial necrosis and the association of PAPP-A levels to the presence of risk factors for coronary artery disease was also studied. We measured PAPP-A levels in patients with AMI (30), UA (23) and asymptomatic controls (45). PAPP-A was estimated using PAPP-A US (ultra sensitive) ELISA manufactured by DRG (Germany). PAPP-A levels were significantly elevated in patients with AMI and in patients with UA (mean levels 64.26 ± 1.05 and 36.23 ± 1.05 ng/ml respectively; $p < 0.001$). Mean PAPP-A levels in controls were 10.68 ± 1.04 ng/ml. In UA cases PAPP-A levels were elevated when the troponin I and CK-MB levels were within the normal range. No correlation was

observed between PAPP-A with markers of myocardial necrosis. PAPP-A can serve as a useful biomarker in the diagnosis of ACS, especially UA, where cardiac troponin levels and CK-MB levels are not elevated and ECG changes are inconclusive.

Keywords PAPP-A · Acute coronary syndrome · Biomarker

Introduction

Pregnancy associated plasma protein-A (PAPP-A) was initially identified as a circulating protein in the serum of pregnant women. It is a zinc binding metalloproteinase used for screening of Down's syndrome during pregnancy. Recent studies have suggested that PAPP-A is an emerging biomarker in the group of markers of inflammation and plaque instability [1–3]. Bayes-Genis et al. [4], described PAPP-A as a potent marker for coronary artery disease (CAD) and acute coronary syndrome (ACS), by demonstration of elevated levels of PAPP-A in unstable plaques. It is the uncomplexed form of PAPP-A that is elevated during ACS. Binding of pro major basic protein to PAPP-A molecule renders it inactive. Unbound PAPP-A acts as a metalloproteinase, increased activity of which causes rupture of the plaques [4].

The present study aimed to evaluate this novel biomarker in the setting of ACS.

Methods

The study was carried out at the Department of Biochemistry and Clinical Lab, Amrita Institute of Medical Science,

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Kochi, India. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all the patients.

The study sample consisted of three groups: 30 patients diagnosed of acute myocardial infarction (AMI) (mean age 62.23 ± 8.62 years and 80 % males); 23 patients diagnosed of unstable angina (UA) (mean age 56.57 ± 8.98 years and 82.6 % males) and 45 controls (mean age 52.33 ± 7.36 years and 53.3 % males). The exclusion criteria considered during the selection of the study population were chronic renal failure, hepatic disorders, connective tissue disorders and valvular heart diseases. Pregnant women were excluded from all the study groups.

Venous blood was drawn from patients, under strict aseptic conditions, admitted from emergency department, at the time of admission.

Similarly fasting blood samples were obtained from patients for analysis of lipid profile and glycemic status.

Venous blood was collected in red vacutainers (without anticoagulant) for measurement of PAPP-A, lipid profile and creatine kinase MB (CK-MB). Troponin I was measured in plasma collected in violet vacutainers containing EDTA. Blood sample for measurement of blood sugar was collected in grey vacutainers, containing sodium fluoride.

Serum was obtained after centrifugation of the venous blood, and stored at -20°C until analysis of PAPP-A, while lipid profile and glycemic status was measured and assessed the same day as collection of sample.

Blood pressure was recorded at the time of clinical examination.

Levels of PAPP-A was determined using DRG PAPP-A ultra sensitive (US) Enzyme Immunoassay Kit (Germany), according to the manufacturer's instructions. The range of the assay was between 0 and 450 ng/ml. The expected value for healthy individuals was <23.14 ng/ml (5–95 %). The analytical sensitivity of the kit was 0.023 ng/ml. The intra assay coefficient of variation (CV%) at 173.2 and 11.96 ng/ml ($n = 20$) was 4.27 and 6.86 % respectively. The corresponding inter assay coefficients of variation ($n = 20$) was 5.86 and 9.4 % respectively.

Lipid profile and fasting blood glucose was measured by colorimetry (OLYMPUS).

The concentration of troponin I and creatine kinase MB fraction (CK-MB) was determined by chemiluminescence (ARCHITECT) and immunoturbidimetry (OLYMPUS)

respectively. Troponin I levels above 0.4 ng/ml and CK-MB levels above 24 U/l were considered positive.

Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics 20 Windows (SPSS Inc., Chicago, USA). For all the continuous variables, the results are either given in mean $\pm$ SD or in median (range) and for categorical variables as percentage. As there was significant difference in age and gender, among the study samples, analysis of covariance (ANCOVA) was applied. To compare the averages of continuous variables between three groups, for those following normal distribution, ANOVA was applied followed by multiple comparison by Bonferroni test, while, for those which were not following normal distribution, Kruskal–Wallis Test was performed. For Multiple comparison Mann–Whitney *U* test was used. Spearman's rho correlation coefficient was used for finding the association between two continuous variables. The $p < 0.05$ were considered as statistically significant.

Results

The demographic, biochemical and risk-factor profiles of the study sample are shown in Tables 1, 2 and 3.

In the study sample, the patients diagnosed of AMI and UA comprised of 80 and 82.6 % males, respectively. Among the controls, 53.3 % were males. There was a significant difference between the number of males and females in the study. Similarly, a significant difference in age of the patients in each group was observed. The significant difference in age and gender was considered when calculating mean and standard deviation of PAPP-A levels in each study population, hence ANCOVA was applied. Age and gender adjusted mean of levels of PAPP-A was assessed. The mean PAPP-A levels in each group (after age and gender adjustment) showed significant difference ($p < 0.001$) (Table 4; Fig. 1). Hence multiple comparison (Bonferroni Test) was applied. Multiple comparison (Bonferroni test) of the mean PAPP-A levels showed that there was significant difference in the mean value between all three groups. PAPP-A was significantly elevated in patients with AMI when compared with PAPP-A levels in patients with UA and asymptomatic controls. Similarly, the

Table 1 Demographic profile of the study sample

Group	AMI	UA	Controls	<i>p</i> value
Age (Mean $\pm$ SD)	62.23 ± 8.62	56.57 ± 8.98	52.33 ± 7.36	<0.001
Males (%)	80	82.6	53.3	<0.001

PAPP-A levels in patients with UA were significantly elevated than PAPP-A levels in asymptomatic controls. There was a significant difference between the mean of PAPP-A, troponin I and CK-MB in the patients with AMI and patients with UA as well as controls (Table 5). Multiple comparison of mean troponin I levels (Mann–Whitney *U* test) and CK-MB levels (Bonferroni Test) showed a significant difference between patients with AMI compared to patients with UA and controls. Correlation of PAPP-A with troponin I and CK-MB was analysed using spearman's correlation coefficient. A very low positive correlation was observed between PAPP-A levels with troponin I and CK-MB in patients with AMI. However, it was not statistically significant.

In patients with UA, no correlation was observed between PAPP-A levels and levels of troponin I. A very low positive correlation was seen between levels of PAPP-A and CK-MB in these patients.

The biochemical profile showed significant difference in serum levels of HDL and diastolic and systolic blood pressure.

Discussion

The diseases of the heart are the most common cause of death throughout the world and early diagnosis of the cardiac dysfunction is a major thrust area of medical research. The natural history of CAD is complex and is characterized by the central role played by inflammation. Oxidized lipoproteins, glycation end products, hypertension, and smoking, all are atherogenic stimuli, which cause endothelial dysfunction and apoptosis, resulting in vascular

Table 3 Incidence of risk factors for CAD in the study sample

Risk factor	AMI (30) (%)	UA (23) (%)	Controls (45) (%)	<i>p</i> Value
Diabetes mellitus	50	43.5	37.8	0.58
Dyslipidemia	86.7	91.3	71.1	0.08
Hypertension	76.7	47.8	46.7	0.02
h/o CAD	46.7	34.8	11.1	0.002

Table 4 Mean PAPP-A levels in each group

Group	PAPP-A (ng/ml) mean $\pm$ SD	Age and gender adjusted mean PAPP-A mean $\pm$ SD	<i>p</i> value
AMI	67.42 $\pm$ 1.16	64.26 $\pm$ 1.05	<0.001
UA	36.81 $\pm$ 1.16	36.23 $\pm$ 1.05	
Control	10.26 $\pm$ 1.44	10.68 $\pm$ 1.04	

expression of adhesion molecules, and recruitment of inflammatory cells [5]. Disruption of an atherosclerotic plaque with subsequent thrombosis is the most important pathogenetic mechanism for ACS [6]. It has been suggested that as a metalloproteinase, PAPP-A, produced by activated macrophages, may degrade the plaque extracellular matrix resulting in consequent weakening of the fibrous cap. This might produce a highly vulnerable plaque that is prone to rupture [7]. The utility of PAPP-A as a marker to detect plaque instability and thus its role as an early marker for identification of patients with acute events of CAD was studied here.

Table 2 Biochemical profile of the study sample

Lab parameter	AMI	UA	Controls	<i>p</i> Value
Fasting blood sugar (mg/dl)	112.63 $\pm$ 30.46 101.45 (71.4–205) ^a	111.31 $\pm$ 45 100.5 (72–303) ^a	105.16 $\pm$ 31.15 90.2 (77.4–192.6) ^a	0.08
Total cholesterol (mg/dl)	193.55 $\pm$ 39.14 203.15 (79–238) ^a	191.61 $\pm$ 44.76 201.8 (106–311.5) ^a	199.57 $\pm$ 34.19 200 (144.3–286.1) ^a	0.76
HDL (mg/dl)	31.84 $\pm$ 6.38 31.65 (13.5–48.1) ^a	35.49 $\pm$ 10.17 31.2 (25–60.9) ^a	43.79 $\pm$ 8.20 45 (29.1–63.7) ^a	<0.001
LDL (mg/dl)	127.7 $\pm$ 25.78 130.1 (47–170.7) ^a	125.12 $\pm$ 33.75 120.6 (67.1–213.8) ^a	135.14 $\pm$ 26.42 130.4 (95.3–183.9) ^a	0.39
TG (mg/dl)	140.62 $\pm$ 61.5 128.55 (63.1–389.6) ^a	119.43 $\pm$ 37.25 113.5 (63.5–211.6) ^a	130.41 $\pm$ 58.14 124.6 (57–373) ^a	0.41
Diastolic blood pressure	93.07 $\pm$ 6.8 90 (72–110) ^a	89.57 $\pm$ 9.76 90 (70–100) ^a	87.11 $\pm$ 5.48 90 (70–100) ^a	0.001
Systolic blood pressure	144.17 $\pm$ 7.2 140 (130–160) ^a	132.65 $\pm$ 14.70 140 (100–180) ^a	133.11 $\pm$ 7.33 130 (120–150) ^a	<0.001

^a Median (range)

Fig. 1 Mean PAPP-A levels in each group

In our study, the circulating concentrations of PAPP-A in serum of patients with UA and AMI were highly elevated than the patients without CAD. The mean PAPP-A levels were 64.26 ± 1.05 , 36.23 ± 1.05 and 10.68 ± 1.04 ng/ml in patients diagnosed of AMI and UA and in controls respectively.

Multiple comparison (Bonferroni test) of the mean PAPP-A levels showed that there was significant difference in the mean value between all three groups. PAPP-A was significantly elevated in patients with AMI when compared with PAPP-A levels of patients with UA and asymptomatic controls. Similarly, the PAPP-A levels in patients with UA were significantly elevated than PAPP-A levels in asymptomatic controls (p value $0 < 0.001$).

Bayes-Genis et al. [4] had first described the presence of increased PAPP-A concentration in patients suffering from UA and ACS. Similarly, Heeschen et al. had observed raised PAPP-A levels in ACS than stable angina and patients without evidence for CAD, while Iversen et al. demonstrated significantly higher PAPP-A levels in patients with STEMI than those with NSTEMI and UA, at the time of admission [8, 9].

Elesber et al. [10] reported a significant difference in mean PAPP-A levels between non cardiac chest pain and ACS patients, confirming the role of PAPP-A in acute coronary events. In a case control study by Mahto et al. [11], PAPP-A was found to be important biomarker to

discriminate cases of MI from UA and controls after using discriminate analysis.

According to studies of Lund et al. [12], PAPP-A concentration measurement was useful for the detection of unstable vascular disease even in patients who do not show increased concentrations of myocardial necrosis biomarkers, troponins and CK-MB. In both the above studies, the correlation of PAPP-A and cTnl levels was weak, thus disqualifying this enzyme as a potential myocardial necrosis marker.

Our study results also demonstrates similar findings. There was a significant difference between the mean of PAPP-A, troponin I and CK-MB in the patients with AMI and patients with UA. PAPP-A levels were significantly elevated in patients with low troponin I and CK-MB levels (UA). Only a mild positive correlation could be seen between PAPP-A levels with CK-MB in these patients. These findings thus support the previous studies implying that PAPP-A is released in circulation earlier than troponin I and CK-MB, and thereby confirms that PAPP-A is a marker of plaque instability, released into circulation before myocardial necrosis.

In our study sample, there was a high incidence of dyslipidemia among all three groups. A significant difference was observed in the mean PAPP-A levels of controls with dyslipidemia and hypertension when compared to controls without these risk factors. (12.16 ± 2.92 vs 7.85 ± 2.63 ; $p < 0.001$ and 12.32 ± 2.37 vs 9.69 ± 3.78 ; $p < 0.05$ respectively). No significant difference was observed in the mean PAPP-A levels among diabetic and non diabetic patients of all three groups, while a significant elevation of PAPP-A levels was seen in patients diagnosed of UA with a positive history of CAD, when compared to patients diagnosed of UA, but no previous history of CAD ($p < 0.05$).

To conclude about the influence of risk factors on PAPP-A levels in the circulation, long term studies on a larger population are required.

Our study results have shown PAPP-A levels are elevated in cases of both AMI and UA, when compared to asymptomatic controls, thus proving PAPP-A to be a marker in ACS. Moreover, elevation of PAPP-A levels in cases of UA, when myocardial necrosis markers are within

Table 5 Markers of myocardial necrosis and PAPP-A levels

Marker	AMI	UA	Controls	<i>p</i> value
PAPP-A (ng/ml) ^a	64.26 ± 1.05	36.23 ± 1.05	10.68 ± 1.04	<0.001
Troponin I (ng/ml) ^b	19.11 ± 18.21	0.062 ± 0.096	0.01 ± 0.009	<0.001
CK-MB (IU/L) ^a	62.23 ± 39.82	18.89 ± 7.09	13.99 ± 3.6	<0.001

^a ANOVA

^b Kruskal–Wallis test

the normal range and ECG changes are inconclusive, highlights the utility of PAPP-A in the early diagnosis of ACS.

However, further larger clinical trials would help in enhancing the diagnostic capability of this novel biomarker.

A limitation in the estimation of PAPP-A is the standardization of assays due to the variation of complexed/uncomplexed PAPP-A epitopes. Ultra-sensitive assays have been introduced but these assays are not equivalent in diagnostic value in non-pregnant patients. Utility of PAPP-A as a clinical cardiac biomarker will require assay standardization and finding an optimal cut-off accordingly in addition to further clinical investigations. Elucidation of the actions of PAPP-A in the unstable plaque may hold the promise of leading to the development of specific plaque-directed therapies for the treatment of both stable and unstable coronary syndromes [13].

Conclusion

The estimation of PAPP-A along with regular markers of myocardial necrosis in patients suspected of ACS might prove to be an important tool for diagnostic and therapeutic stratification of patients with ACS without evidence for myocardial necrosis.

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