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Original article

Pregnancy associated plasma protein-A (PAPP-A) as an early marker for the diagnosis of acute coronary syndrome

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KEYWORDS

Acute coronary syndrome-Biomarker
Atherosclerosis
PAPP-A-metalloproteinase

ABSTRACT

Aims and objectives: Pregnancy associated plasma protein-A (PAPP-A), a metalloproteinase plays a pivotal role in the pathogenesis of atherosclerosis. Recent studies have reported that elevated levels of PAPP-A, signal the onset of acute coronary syndrome (ACS). We, therefore, proposed to study the analytical competence of PAPP-A in patients admitted to the emergency department with chest pain and finally diagnosed as ACS.

Methods and results: Pregnancy associated plasma protein-A was measured using enzyme-linked immunosorbent assay (ELISA) in 485 patients admitted to emergency care unit, of which 89 patients were diagnosed as Non-cardiac chest pain (NCCP). Elevated levels of PAPP-A were observed in patients diagnosed as ACS on comparison with the controls. Receiver operator characteristic (ROC) curve analysis showed PAPP-A to be a good discriminator between ischaemic and non-ischaemic patients. The area under the curve was found to be 0.904, 95% CI (0.874–0.929) with 90% sensitivity and 85% specificity ($P<0.0001$). The cut-off value from the ROC curve was 0.55 μg/mL above which PAPP-A was considered to be positive.

Conclusion: Pregnancy associated plasma protein-A seems to be a promising biomarker for identification and risk stratification for patients with ACS.

Introduction

Diagnosis of patients with acute coronary syndrome (ACS) is a major challenge for the clinicians at the emergency unit. Despite the success of cardiac troponins as gold standard, the search for a biomarker which precedes necrosis is still on. Recent studies have suggested that pregnancy associated plasma protein-A (PAPP-A) is an emerging biomarker in the category of markers of inflammation and plaque instability.[1–3] Pregnancy associated plasma protein-A is a zinc binding metalloproteinase which was originally identified as a circulating protein in the serum of women in advanced stages of gestation. In 2001, Bayes-Genis et al[4] described PAPP-A as a potent marker for coronary artery disease (CAD) and ACS. The demonstrated elevated levels of PAPP-A are found in unstable plaques than in stable plaques. Later, several studies have reported that circulating (elevated) levels of PAPP-A, independently predict the risk of an ischaemic event.[5,6] Uncomplexed form of PAPP-A is elevated in ACS. Binding of pro major basic protein (MBP) to PAPP-A molecule is hindered due to oxidative stress in the microenvironment of the plaque. Unbound PAPP-A acts as a metalloproteinase causing rupture of the plaques. To the best of our knowledge, no study demonstrating PAPP-A as a sensitive biomarker for ACS in Indian population has been carried out. Therefore, we evaluated the circulating PAPP-A concentrations to detect the onset of ACS.

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Methods

Patients

The study was carried out at the Department of Biochemistry and Clinical Lab, International Centre for Cardiothoracic and Vascular Diseases, Frontier Lifeline & Dr. KM Cherian's Heart Foundation, Chennai, India. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all the patients.

The study group included 485 consecutive patients admitted to critical care unit (CCU) with manifestations, suggestive of acute myocardial ischaemia including those with chest pain with or without radiation, compressing chest discomfort, palpitations, shortness of breath, lower jaw pain, left arm pain, epigastric pain, hypotension and other symptoms suggestive of angina. 12-lead electrocardiogram and all demographic details of the patients were recorded. Of the 485 consecutive patients admitted to CCU, 297 patients had ACS (98 patients with ST-segment elevation, 99 patients without ST-segment elevation and 100 patients with unstable angina) with a mean age of 55±11 years and 76% of them being males. Remaining patients were diagnosed as non-cardiac chest pain (NCCP) with a mean age of 52±11 years and 67% being males. Among 99 healthy volunteers with no clinical evidence of heart disease, a mean age of 50±12 years and proportion of males being 63% were observed. Patients with liver, kidney disorders, brain ischaemia, tumour, and pregnant women were excluded from the study.

Blood sampling

Venous blood was drawn from patients admitted to CCU within 4–6 hours after symptom onset into plain tubes (without anticoagulants) and allowed to clot for half-an-hour, before centrifugation. Serum was separated and stored at –40°C until analysis and the samples were thawed only once.

Detection of pregnancy associated plasma protein-A, troponin I, and creatine kinase-MB fraction

Levels of PAPP-A were determined using Demeditec Diagnostics (Germany), according to the manufacture's instructions. The concentrations of troponin I and creatine kinase-MB fraction (CK-MB) were determined by microparticle enzyme immunoassay (MEIA) (Abott Axsym) and International Federation of Clinical Chemistry (IFCC) methods respectively. Troponin I levels above >0.1 ng/mL and CK-MB levels >25 U/L were considered to be positive.

Statistical analysis

Statistical evaluations were performed using Statistical Package for the Social Sciences (SPSS) software 9.0. Receiver operator characteristic curve analysis was performed using MedCalc 9.6. Data are expressed as mean±SD. Significance between subgroups were analysed using Krushkal Wallis test. P values <0.05 were considered to be statistically significant.

Results

The biochemical, haematological parameters and risk factors of the study groups are summarised in Table 1. Mean PAPP-A values (μg/mL) in the ST-segment elevated myocardial infarction (STEMI), non-STEMI (NSTEMI) and unstable angina (UA) patients were 1.13±0.25, 0.90±0.29, and 0.84±0.26, respectively (Table 2). The differences were found to be significantly higher in patients than in controls (0.31±0.33) (P<0.001). The levels in NCCP were (0.43±0.49) (P<0.001) also found to be lower in comparison with patients. Figure 1 depicts the levels of mean PAPP-A in different study groups. Figure 2 shows the PAPP-A values in cases and controls. Intercomparison of groups revealed no significant differences between controls and NCCP & STEMI and NSTEMI. All other group comparisons were found to be highly significant (P<0.001).

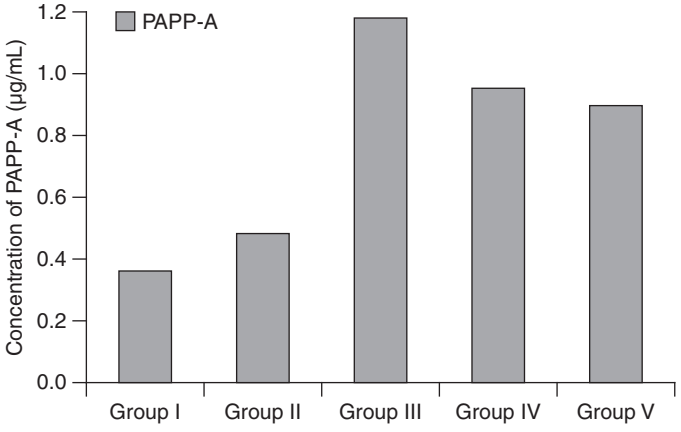

Figure 1 Mean pregnancy associated plasma protein-A (PAPP-A) levels in differenct study groups.

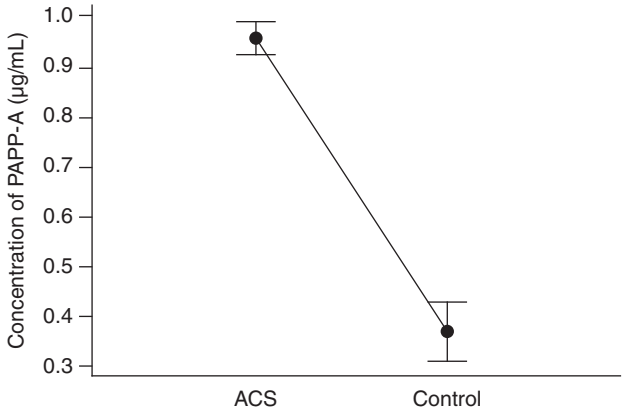

Figure 2 Pregnancy associated plasma protein-A (PAPP-A) levels in acute coronary syndrome (ACS) and control.

Table I
Baseline characteristics of patients with acute coronary syndrome and control subjects.

Parameter	Control (n=99)	NCCP (n=89)	STEMI (n=98)	NSTEMI (n=99)	UA (n=100)	P value
Age (yr)	45±12	53±12	55±12	56±13	55±11	<0.001
Male (n)	63	67	89	71	67	<0.001
Serum glucose (mg/dL)	108.34±23.93	142.18±47.77	214.54±91.88	170.27±75.03	184.23±81.34	<0.001
Serum total cholesterol (mg/dL)	168.8±18.39	170.18±23.56	186.19±40.86	194.23±43.11	183.8±39.24	<0.001
Serum triglycerides (mg/dL)	148.14±33.20	148.95±45.87	157.67±63.20	151.26±53.17	157.49±67.10	NS
Serum LDL-cholesterol (mg/dL)	82.04±24.07	109.16±29.94	118.23±34.96	118.97±37.82	126.86±39.77	<0.001
Serum HDL-cholesterol (mg/dL)	47.42±7.86	38.02±5.60	37.89±5.77	37.41±4.47	37.31±3.70	<0.001
Serum urea (mg/dL)	26.76±5.06	25.77±4.27	27.49±4.77	27.35±4.67	25.33±4.67	NS
Serum creatinine (mg/dL)	0.83±0.14	0.80±0.11	0.84±0.14	0.82±0.13	0.81±0.11	NS
Serum total protein	6.04±0.76	5.94±1.01	6.06±0.67	6.09±0.91	6.05±0.84	NS
Total WBC count (cells/cmm)	7812.36±1488.50	8224.44±1890.94	11783.67±3645.91	9676.77±3205.53	9771.00±2857.78	<0.001
Haemoglobin (g/dL)	14.09±1.58	13.66±1.60	14.16±1.55	13.66±1.54	13.55±1.52	NS
Diabetes mellitus (%)	20	36	61	58	62	<0.001
Hypertension (%)	2	21	47	54	55	<0.001
Family history of CAD (%)	19	15	46	48	49	<0.001
Smoking (%)	21	35	41	40	27	<0.001
Food habits (non-vegetarian) (%)	56	79	77	66	76	<0.001
Diastolic blood pressure (mmHg)	79.95±1.13	83.71±8.97	87.15±13.75	88.01±13.49	87.40±12.51	<0.001
Body mass index (kg/m^2)	25.79±3.82	26.05±3.54	25.84±4.95	25.39±3.56	27.06±3.60	<0.001

Data are expressed as mean±SD for continuous variables and percentage (%) for categorical variables. CAD: coronary artery disease, HDL: high density lipoprotein, LDL: low density lipoprotein, NCCP: non-cardiac chest pain, NS: non-significant, NSTEMI: non-ST segment elevation myocardial infarction, STEMI: ST segment elevation myocardial infraction, UA: unstable angina, WBC: white blood cell.

Table 2
Serum levels of pregnancy associated plasma protein-A in the sub groups of acute coronary syndrome on comparison with goal standards.

Parameter	Control	NCCP	STEMI	NSTEMI	UA	P value
PAPP-A (μg/mL)	0.31±0.33	0.43±0.49	1.13±0.25	0.90±0.29	0.84±0.26	<0.001
Troponin I (μg/mL)	0.02±0.04	0.84±0.42	6.65±8.30	2.34±5.46	0.44±2.23	<0.001
CK-MB (IU/L)	19.47±7.31	20.17±8.56	94.24±87.84	62.61±74.53	29.63±25.00	<0.001

CK-MB: creatine kinase-MB fraction, NCCP: non-cardiac chest pain, NSTEMI: non-ST-segment elevation myocardial infarction patients, PAPP-A: pregnancy associated plasma protein-A, STEMI: ST-segment elevation myocardial infarction patients, UA: unstable angina patients.

Table 3
Sensitivity, specificity, area under the curve and 95% confidence interval of Troponin I, creatine kinase-MB fraction and pregnancy associated plasma protein-A at the optimum cut-off value obtained from the receiver operator characteristic curve.

Biomarker	Sensitivity (%)	Specificity (%)	AUC	95% CI
Troponin I	54	95	0.765	0.725–0.802
CK-MB	57	93	0.798	0.760–0.833
PAPP-A	90	85	0.904	0.874–0.929

CI: confidence interval, CK-MB: creatine kinase-MB fraction, AUC: area under the curve, PAPP-A: pregnancy associated plasma protein-A.

From the ROC curve, the optimum cut-off value for PAPP-A was found to be 0.55 μg/mL (Figure 3). The area under the curve was found to be 0.904, 95% CI (0.874–0.929) (P<0.0001) with 90% sensitivity and 85% specificity (Table 3). Figure 4 shows the comparison of ROC curves of PAPP-A with gold standards namely troponin and CK-MB, and PAPP-A was found to be highly significant.

Discussion

Pregnancy associated plasma protein-A, a zinc binding metalloproteinase is abundantly expressed in plaque cells and extracellular matrix of eroded and ruptured plaques.[4] During the evaluation of unstable plaques in patients who have died suddenly of cardiac causes, an association between PAPP-A and atherosclerotic plaque was confirmed using histological evidence. Pregnancy associated plasma protein-A was originally used to determine the foetal diagnosis of Down syndrome. However, circulating concentrations of PAPP-A were shown

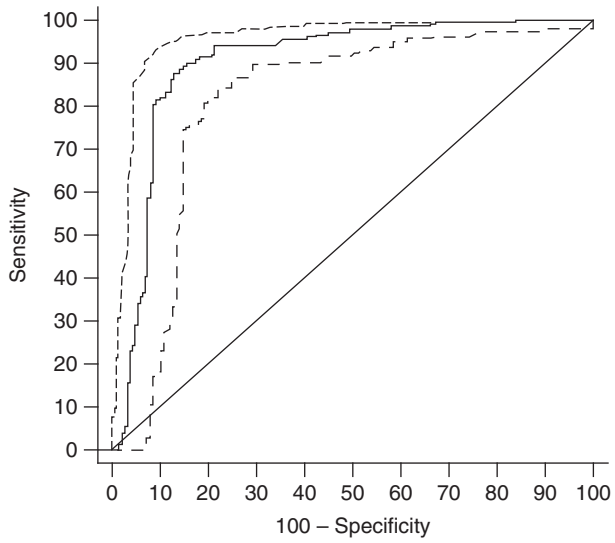

Figure 3 Receiver operator characteristic curve analysis for the assay of pregnancy associated plasma protein-A.

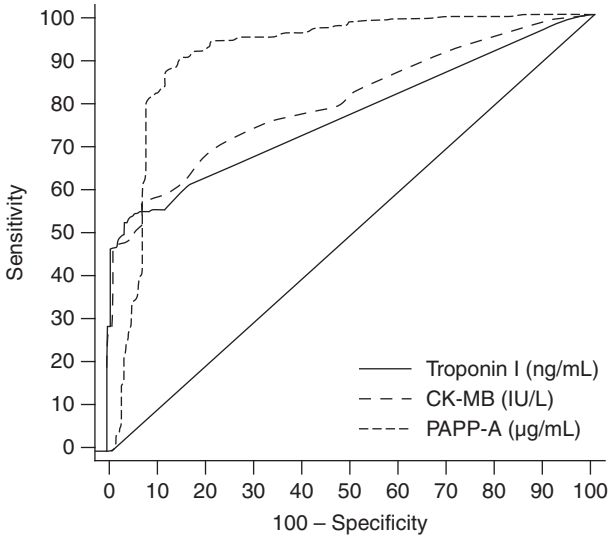

Figure 4 Comparison of the receiver operator characteristic curves. CK-MB: creatine kinase-MB fraction, PAPP-A: pregnancy associated plasma protein-A.

to be present in lower concentrations in both men and non-pregnant women.

Our study results show that circulating concentrations of PAPP-A in serum of patients with both UA and acute MI were higher than controls and NCCP. These were in agreement with Bayes-Genis et al.[7] who observed increased PAPP-A concentrations even in patients with negative cardiac troponin. Pregnancy associated plasma protein-A appeared to be an independent predictor of future ischaemic events as well as the need for percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery.[5]

Similar findings were reported by Heeschen et al.[6] who observed raised PAPP-A in ACS than stable angina and patients without evidence for CAD. Mean PAPP-A levels in NSTEMI patients did not significantly differ from mean PAPP-A levels in UA.

The current study also observed no significant difference between NSTEMI and UA. But STEMI levels were found to be elevated than NSTEMI and UA. Similar results were observed by Iversen et al.[8] where PAPP-A levels at admission were significantly higher in patients with STEMI than those with NSTEMI and UA. Elseber et al.[9] reported a significant difference in mean PAPP-A levels between NCCP and ACS patients which were in very good agreement with our results. But there was an overlap in the serum PAPP-A levels due to a very small sample size in the study by Elseber et al.[9] Prior reports, however, have clearly documented the prognostic utility of PAPP-A levels in similar populations. Iversen et al.[10] have reported low levels of PAPP-A in patients without heart disease and confirm the association of elevated PAPP-A levels with the diagnosis of ACS. Our results also showed a significant difference between the mean PAPP-A levels in ACS patients when compared to NCCP.

Receiver operator characteristic curve analysis of our study population reported first in Indian population showed the area under the curve to be 0.904 with 90% sensitivity and 85% specificity (Table 2) at a cut-off value of 0.55 μg/mL. In patients with samples drawn <6 hours after the onset of symptom, PAPP-A seemed to be more sensitive than CK-MB and troponin.[8] Our results are highly significant when compared with the study by Laterza et al.[11] who reported a cut-off value of 0.22 mIU/L, with a sensitivity of 66.7% and a specificity of 51.1%.

Laterza et al.[11] concluded PAPP-A to be a modest predictor of adverse events at 30 days. Heeschen et al.[6] in a similar study showed PAPP-A was a powerful predictor both in patients with low and high troponin levels.

Other studies have demonstrated that in asymptomatic male subjects whose carotid intima media thickness and lesion status were evaluated by non-invasive ultrasonography, the presence of hyperlipidaemia and hyperechoic or isoechoic and echogenic lesions were associated with significantly higher PAPP-A levels.[12]

The exact role of PAPP-A in the pathophysiology of PAPP-A remains unclear but PAPP-A activates insulin-like growth factor-I (IGF-I), a potent mediator of atherosclerosis.[13] Pregnancy associated plasma protein-A may be involved in the process of plaque rupture and destabilization and hence plays an important role as significant predictive factor for diagnosis of patients presenting to CCU with acute chest pain.[14] Furthermore, the prior reports have suggested the use of serum samples for PAPP-A to be highly beneficial than other sample types.[15]

In a substudy of the CAPTURE trial, elevated PAPP-A levels (>12.6 mIU/L) in patients with ACS indicated an increased risk of death or MI at 30 days and 6 months, even in patients with negative troponin results.[6] To date, the main problem is the standardisation due to the variation of complexed/uncomplexed PAPP-A epitopes. Ultra-sensitive assays have been identified but these assays are not equivalent in diagnostic value in non-pregnant patients. Adoption of PAPP-A as a clinical cardiac biomarker will require assay standardisation

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.[1]

In conclusion, PAPP-A elevations in circulation plays an important role in the diagnosis of ACS. Although, the study has been conducted on a reasonably good sample size, it is felt that further larger clinical trials would enhance the diagnostic capability of this novel biomarker.

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