

Original research article

**A STUDY ON PREGNANCY ASSOCIATED PLASMA
PROTEIN A (PAPP-A) AS A PREDICTOR OF ACUTE
CORONARY SYNDROME IN PATIENTS PRESENTING
WITH CHEST PAIN TO A TERTIARY CARE HOSPITAL**

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Abstract

Background: Acute coronary syndrome (ACS) is a leading cause of morbidity and mortality worldwide. Early detection is crucial for reducing cardiac damage in people with ACS. Prompt risk classification and diagnosis allows for early therapy commencement, reducing the patient's chance of unfavourable outcomes. Cardiac troponins I and T, which have high sensitivity and tissue specificity, are often used to diagnose ACS alongside an electrocardiogram (1).

Objectives:

1. Study the analytical competence of PAPP-A in patients admitted to the emergency department with chest pain and finally diagnosed as ACS.
2. Comparison of PAPP-A levels in sub-types of ACS.
3. Comparison of PAPP A and troponin I in subtypes of ACS.

Material & Methods:

Study Design: Case control study.

Study area: The study was conducted in the Department of General Medicine, in a tertiary Care teaching hospital.

Study Period: October 2022 to September 2023.

Sample Size: A case-control study of 88 subjects divided equally into 2 groups.

Study Tools and Data Collection Procedure

Specimen Collection: 5 ml venous blood was drawn from all groups within 6 hours of

presentation to emergency department, in red capped tubes. Blood was allowed to clot and serum separated thereafter. Hemolysed and lipemic samples were not accepted. Serum was stored in aliquots at -20°C for estimation of PAPP A and Troponin I.

Parameters Estimated: Serum:

- 1) PAPP A.
- 2) Troponin I.

Results: In this study, the mean serum levels of Troponin-I in UA, NSTEMI and STEMI are $2.306 + 1.83$, $11.29 + 7.84$, $38.95 + 16.86$ respectively. Among 50 patients of acute coronary syndrome, suggesting that Troponin levels are more in STEMI, NSTEMI, UA patients compared to normal controls. Troponin-I levels are significantly higher in blood samples from patients diagnosed with MI. The mean serum PAPP-A levels are $0.113 + 0.3006$, $0.294 + 0.393$, $1.379 + 1.66$ in UA, NSTEMI and STEMI patients whereas mean serum levels of Troponin-I are $2306 + 1.8$, $11.29 + 7.849$ and $38.95 + 16.86$ in UA, NSTEMI and STEMI patients.

Conclusion: PAPP-A might be useful as an indicator of inflammation to identify unstable plaque and might, consequently, be an important clinical aid in reducing the incidence of ACS and the disability and mortality associated with this condition. Circulating PAPP-A levels can identify patients early in the process of plaque instability, when it might still be possible to avert myocardial injury.

Keywords: Acute coronary syndrome, cardiac troponins, PAPP-A, disability and mortality

Introduction

Acute coronary syndrome (ACS) is a leading cause of morbidity and mortality worldwide. Early detection is crucial for reducing cardiac damage in people with ACS. Prompt risk classification and diagnosis allows for early therapy commencement, reducing the patient's chance of unfavourable outcomes. Cardiac troponins I and T, which have high sensitivity and tissue specificity, are often used to diagnose ACS alongside an electrocardiogram ^[1].

Cardiac troponins are limited in their ability to detect myocardial necrosis because they are only released into the bloodstream after heart muscle damage occurs. Previous approaches required many hours after symptom onset to detect increases ^[2]. Cardiac troponin assays have improved in sensitivity over time. This allows for early detection of smaller elevations in ACS ^[3].

On the other side, this has caused issues in patient care ^[4]. Sensitive assays detect cardiac troponin increases in a wider range of individuals, including those who may not require severe therapy. Mild troponin elevations can indicate myocardial injury, but they may also be associated with other diseases that do not pose a high risk of irreversible cardiac outcomes ^[5].

Recent cardiovascular disease research aims to identify biomarkers that present in the bloodstream prior to substantial myocardial necrosis. These markers are expected to improve the diagnosis and risk classification of patients with ACS symptoms including chest pain and shortness of breath when they arrive at the emergency unit. The new markers aim to improve risk prediction, particularly for patients lacking cardiac troponin elevations upon admission. The new markers can help manage patients with minor cardiac troponin increases assessed by sensitive assays, reducing the requirement

for intensive treatment with higher risk.

Several substances have been identified as potential contributors to the pathogenic processes that cause ACS. These indicators may indicate inflammation, plaque instability, rupture, thrombosis, ischemia, and myocardial dysfunction^[6, 7]. PAPP A, a marker associated with pregnancy, has recently gained attention as a potential option. PAPP-A expression in arterial smooth muscle inhibits vascular responsiveness to damage *in vivo*. Elevated PAPP A levels can detect the beginning of ACS prior to infarction^[8]. The study aimed to determine if PAPP-A is an early diagnostic marker for acute coronary syndrome in patients experiencing chest pain.

Objectives

1. Study the analytical competence of PAPP-A in patients admitted to the emergency department with chest pain and finally diagnosed as ACS.
2. Comparison of PAPP-A levels in sub-types of ACS.
3. Comparison of PAPP A and troponin I in subtypes of ACS.

Material & Methods

Study Design: Case control study.

Study Area: The study was conducted in the Department of General Medicine.

Study Period: April 2022 to March 2023.

Sample Size: A case-control study of 88 subjects divided equally into 2 groups:

Group 1	Acute coronary syndrome patients	50
Group 2	Healthy controls	38

Sampling Technique: Simple Random technique.

Inclusion Criteria:

Group 1:

- Mean age 55 ± 20 years.
- Manifestations s/o acute MI.
- Chest pain, compressing chest discomfort, palpitations, shortness of breath.
- Lower jaw pain, left arm pain, epigastric pain, hypotension, ECG changes suggestive of ST segment elevation MI or Non ST segment elevation MI or Unstable angina.

Group 2:

- Healthy volunteers with no clinical evidence of heart disease, a mean age of 50 ± 20 years.

Exclusion Criteria

- Liver or kidney disorder
- Cerebro vascular accident
- Malignancy
- Pregnancy

Ethical Consideration: Institutional Ethical committee permission was taken prior to the commencement of the study.

Study Tools and Data Collection Procedure

Specimen Collection: 5 ml venous blood was drawn from all groups within 6 hours of presentation to emergency department, in red capped tubes. Blood was allowed to clot and serum separated thereafter. Hemolysed and lipemic samples were not accepted. Serum was stored in aliquots at -20 °C for estimation of PAPP A and Troponin I.

Parameters Estimated: Serum: 1) PAPP A, 2) Troponin I.

Methods: Serum PAPP A:

Method: Enzyme-Linked Immunosorbent Assay (ELISA).

Standardization of PAPP A:

	S0	S1	S2	S3	S4	S5
Conc (µg/ml)	0.0	1.0	2.5	5.0	15.0	30
OD (450nm)	0.18	0.38	0.56	0.83	1.44	1.80

Serum Troponin I:

Method: Enzyme-Linked Immunosorbent Assay (ELISA).

Standardization of Troponin I:

	S1	S2	S3	S4	S5
Conc (ng/ml)	0.0	2.0	7.5	30.0	75.0
OD (450nm)	0.048	0.110	0.307	1.357	2.853

Reference Value: <0.5ng/ml Statistical Analysis:

The data has been entered into MS-Excel and statistical analysis has been done by using IBM SPSS Version 25.0. For categorical variables, the data values are represented in terms of numbers and percentages. The chi-square test was used to assess group association. For continuous variables, mean and standard deviation of the data are displayed. The student’s t- test was used to compare the mean differences between the two groups. All p values less than 0.05 are regarded as statistically significant.

Observations & Results: A total of 88 subjects included in the study. Among them 50 patients were diagnosed as coronary artery disease based on ECG, Cardiac biomarkers (PAPP-A and Troponin-T) and 2D Echocardiography. Among 50 members of ACS patients 20, were diagnosed as STEMI, 15 were diagnosed NSTEMI and 15 were

diagnosed as unstable angina. 38 were age and sex matched healthy controls with no evidence of heart disease, kidney disease, pregnancy liver disease, CAD, CVA and malignancy.

The following parameters are analysed: Serum PAPP-A, Serum Troponin-I. Mean serum levels of PAPP-A and Troponin-I was analysed separately in the two groups. The results obtained for PAPP-A were as follows:

Table 1: PAPP-A in Controls and ACS patients

Parameters	Mean	ISD	SEM
Group - I	0.673	1.221	0.1726
Group - II	0.1025	0.3879	0.064

The levels of PAPP-A in ACS patients is 0.673 + 1.221 whereas the levels of PAPP-A in normal healthy controls is 0.102 + 0.38.

Troponin-I	Mean	ISD	SEM
Group-I	17.61	19.49	3.196
Group-II	0.7025	0.84	0.1049

Mean serum value of Troponin I in ACS patients is 17.61 + 19.44. Whereas Troponin-I in normal healthy controls is 0.702 + 0.84.

Table 3: Mean Serum levels of PAPP-A in subtypes of ACS

	Mean	ISD	SEM
STEM-I	1.379	1.666	0.3726
NSTEMI- II	0.2947	0.393	0.1017
UA III	0.1139	0.3006	0.07762

Levels of PAPP-A and Troponin-I in sub types of ACS were analysed separately in 50 ACS patients.

Table 4: Mean serum levels of Troponin-I in each subtypes of ACS

	Mean	ISD	SEM
STEM-I	38.95	16.86	4.675
NSTEMI-II	11.29	7.849	2.482
UA I	2.306	1.83	0.4891

Discussion

The identification and evaluation of patients with suspected ACS remains a clinical challenge, relying mostly on ECG and serum biochemical markers. However, the sensitivity of ECG diagnosis is only about 49-66%, and there are abnormalities in non-Q waves. ECGs in UA patients are not specific [9]. ECG and serum biomarker results

frequently fail to identify the disease in outpatients and high risk patients, hence it is critical to develop new indicators for identifying individuals with ACS. A biomarker capable of detecting unstable plaque would be extremely useful in clinical practice, as it would allow patients at high risk of getting myocardial infarction to be diagnosed. Plaque rupture and abrupt thrombosis cause a significant reduction in coronary blood flow, and acute inflammation also plays a role in the disease process ^[10].

In this investigation, 50 patients with acute coronary syndrome were examined. Because hypertension, diabetes, dyslipidaemia, smoking, and drinking are risk factors for acute coronary syndrome, a history of these conditions is taken into account, and the prevalence of CAD varies by age and gender.

STEMI: As per the study, among 50 patients, 20 were STEMI patients. In these patients, STEMI is more common in men and women. 17 were men and 3 were women. Mean age group was 50 + 20. Smoking history is present in 15 patients out of 20 and history of alcoholism is present in 18. History of hypertension is present in 20 patients, diabetes in 17 patients and dyslipidaemia in 20 patients. ECG changes was consistent with the criteria of STEMI showing ST evaluation of at least 2 mm in two contiguous precordial leads and 1 mm in two adjacent limb leads in all patients. 2d ECHO showing regional wall motion abnormality of posterior wall (or) anterior wall (or) inferior wall (or) and cardiac biomarkers are elevated in all patients. This is in consistent with various studies showing the association of major cardio vascular risk factors with the development of ACS. Smoking, hypertension, dyslipidemia, and diabetes are demonstrated to be risk factors especially in younger individuals. A half of ACS population in the < 45 years age subgroup possessed all four CV risk factors in one study. These results call for attention and implementation of prevention programmes ^[11].

NSTEMI: In this study, 15 members with diagnosed as NSTEMI. Mean age group is 40 + 15 more common in men than in women history of smoking is present 10 members and history of alcoholism is present 9 members out of 15 patients. Hypertension in 15 patients and diabetes in 13 patients. dyslipidaemia is present in all patients. ECG showing ST-T changes (New and deep T-wave inversions > 0.3 mv) (or) ST segment depressions. Echo showing regional wall motion abnormalities in all cases and all patients have elevated cardiac biomarkers.

Unstable Angina: Another 15 members were diagnosed as unstable angina based on their typical presentation and ECG and Echo changes. Mean age group among these patients is 45+ 15. More common in men than in women history of smoking is present in 12 members and history of alcoholism is present in 5 members out of 15 patients. Hypertension in 12 patients and diabetes in 10 patients. Dyslipidemia is present in all the patients. ECG showing ST-T changes (new and deep T Wave inversion > 0.3 MV) (or) ST-Segment depressions.

Echocardiography was normal in all the patients and there was no regional wall motion abnormalities and biomarkers are elevated in some patients.

PAPP A in Acute Coronary Syndrome: In the present study, mean serum PAPP - A levels are $0.673 + 1.221$ in ACS patients where as a PAPP-A level was $0.102 + 0.38$ in normal subjects. This is in consistent with various previous studies, suggesting the elevation of PAPP-A in acute coronary syndromes. Serum PAPP-A concentrations were significantly higher in patients with myocardial infarction (or) UA than in patients with stable angina. Using a cut-off PAPP-A concentrations of 10 MIU/L, it was possible to identify ACS patients with a sensitivity of 89.2% and a specificity of 81.3%. These results indicate that PAPP-A could be a valuable diagnostic marker for the identification of ACS patients ^[12]. At a cut off value of 2.9 MIU/L elevated serum PAPP-A was an independent predictor of adverse cardiac events with an adjusted risk ratio of 4.6 (95%) confidence interval 1.8-11.8. Thus measurement of PAPP-A potentially has value in identifying high risk ACS patients whose unstable clinical situation might otherwise remain unrecognized ^[13].

Headchen and colleagues studied a heterogenous population of individuals who had arrived at the emergency room with chest pain. PAPP-A concentration was significant higher in those patients who were later diagnosed with ACS. During the index hospitalization than in those who were diagnosed with stable angina (or) who did not have coronary heart disease ^[14]. Similarly, in a study by Elesber and colleagues the admission serum sample PAPP-A concentrations were higher in those intermediate to high risk chest pain patients who were subsequently diagnosed with ACS than in those patients who were diagnosed with non-cardiac chest pain ^[15]. Mean serum levels of PAPP-A in subtypes of ACS.

In this study the mean serum levels of PAPP-A in UA, NSTEMI and STEMI are $0.113 + 0.3006$, $0.294 + 0.393$, $1.379 + 1.66$ respectively, among 50 patients of acute coronary syndrome, suggesting that PAPP -A levels are more in STEMI and NSTEMI than in unstable angina compared to that of normal controls. PAPP-A level was significantly higher in blood samples from patients diagnosed with MI or UA than in samples from normal coronary arteries.

This is in comparison with previous studies, Rossen and Colleagues suggesting that circulating PAPP-A levels were lower in NSTEMI patients than in STEMI patients and yet lower levels are seen in healthy controls ^[16]. Miedema and colleagues reported on higher circulating PAPP-A levels in ACS patients than in patients with stable angina or in asymptomatic CAD patients ^[17]. Lin and colleagues noticed higher serum PAPP-A in STEMI and UA patients than instable angina patients and controls ^[18]. Schoos and colleagues studied high PAPP-A studied high PAPP-A levels in STEMI patients compared to high risk. NSTE ACS patients or low risk NSTE-ACS patients with even lower level ^[19]. Some recent studies by Iversen and colleagues showed higher PAPP-A levels in high risk NSTE-ACS patients than in low risk NSTE-ACS patients ^[20]. More ever the PAPP-A concentrations of this whole patients group were lower than in STEMI patients but higher than in patients with non-cardiac disease or in healthy control whose PAPP-A concentrations had been measured with the same method. McCam and colleagues found higher PAPP-A levels in the admission samples of those patients with acute ischemic type chest pain who were later diagnosed with MI than in those without MI diagnosis ^[21]. The release profile of PAPP-A in ACS has been investigated in some studies. Qin and colleagues (2002) reported on highly variable release profile of PAPP-A in samples serially collected from a peak of elevation was

seen in all patients. However, the time, height and duration of elevation varied remarkably between individuals. No correlation was detected with myoglobin (a marker of myocardial necrosis, but significant association was seen with cTnI ($r=0.419$, $P < 0.001$) [22].

Troponin-I in Acute Coronary Syndrome: In the present study, mean serum Troponin-I levels are 17.61 ± 19.49 in ACS patients whereas Troponin-I levels are 0.7025 ± 0.8 in normal subjects. This was in consistent with various previous studies, suggesting the elevation of PAPP-A in acute coronary syndrome.

Mean Serum Levels of Troponin-I in subtypes of ACS: In this study, the mean serum levels of Troponin-I in UA, NSTEMI and STEMI are 2.306 ± 1.83 , 11.29 ± 7.84 , 38.95 ± 16.86 respectively. Among 50 patients of acute coronary syndrome, suggesting that Troponin levels are more in STEMI, NSTEMI, UA patients compared to normal controls. Troponin-I levels are significantly higher in blood samples from patients diagnosed with MI. The mean serum PAPP – A levels are 0.113 ± 0.3006 , 0.294 ± 0.393 , 1.379 ± 1.66 in UA, NSTEMI and STEMI patients whereas mean serum levels of Troponin-I are 2306 ± 1.8 , 11.29 ± 7.849 and 38.95 ± 16.86 in UA, NSTEMI and STEMI patients.

P value calculated by unpaired t test for PAPP-A is 0.0082. as it is < 0.05 it is considered significant. P value for Troponin-I was < 0.001 . As it was ($p < 0.05$), it is considered significant. This study showed significant elevation of PAPP-A in ACS patients like that of Troponin I. So, PAPP-A is considered as a cardiac biomarker along with troponin I in patients with ACS. It was also found that the serum PAPP-A level was significantly and positively correlated with the serum levels of hs CRP and TNF-alpha in cTnI negative ACS patients, and that raised levels of serum PAPP-A might be useful as an indicator of inflammation to identify unstable plaque and might, consequently, be an important clinical aid in reducing the incidence of ACS [23]. Comparison of PAPP-A in controls and patients of ACS in present study compared to other studies. (By Bayes genes *et al.*) [24].

The predictive value of PAPP-A was also apparent in patients without evidence for myocardial necrosis. TnT-negative patients (threshold level 0.01) with elevated PAPP-A levels were at significantly higher compared with TnI-negative patients with low PAPP-A levels. The predictive value of PAPP-A was also observed for a reduced threshold level of 0.01 ml/L for TnI. Troponin I positive patients suffered from increased cardiovascular risk, and this was particularly true for patients who had elevated PAPP-A levels [25].

Bayer Genis *et al.* observed increased PAPP-A even in patients with negative cardiac troponin patients. PAPP A appeared to be an independent predictor of future ischemic events as well as the need for PCI (or) coronary artery bypass a graft surgery [24]. Laterza *et al.* [26] concluded PAPP-A to be a modest predictor of adverse cardiac events at 30 days. Huschen *et al.* [27] in a sanitary study should PAPP-A was a powerful predictor both in patients with low and high troponin levels.

PAPP-A is considered as a prognostic marker of ACS in some studies and higher levels of PAPP-A were associated with poor prognosis but because of limited sample size and difficulties in follow up, we are not able to show this association. The form of PAPP-A

secreted by vascular cells is a homodimer, not covalently linked with eosinophil major basic protein. This form has proteolytic activity and is considered to play an important role in cardiovascular diseases [28, 29]. Previously, the MERLIN-TIMI-36 study investigator reported that PAPP-A was independently associated with recurrent cardiovascular events in patients with NSTEMI [30]. 89 Wlazel and colleagues examined the clinical values of PAPP-A in predicting future events post ACS and suggested that biomarker can improve the risk prediction [31].

Conclusion

PAPP-A might be useful as an indicator of inflammation to identify unstable plaque and might, consequently, be an important clinical aid in reducing the incidence of ACS and the disability and mortality associated with this condition. Circulating PAPP-A levels can identify patients early in the process of plaque instability, when it might still be possible to avert myocardial injury. 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. Elevation of PAPP-A levels in cases of UA, when myocardial necrosis markers are within the normal range and ECG changes are inconclusive, highlights the utility of PAPP-A in early diagnosis of ACS.

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