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Original Article

Biochemistry Section

Evaluation of Serum Pregnancy Associated Plasma Protein-A & Plasma D-Dimer in Acute Coronary Syndrome

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ABSTRACT

Introduction: Acute coronary syndrome (ACS), a spectrum comprising unstable angina pectoris, ST Elevated Myocardial Infarction (STEMI) & Non ST Elevated Myocardial Infarction (NSTEMI) is the major cause of presentation in Emergency Department today. Though ECG and cardiac enzymes are used for diagnosis, they mislead the diagnosis sometimes and delay in treatment initiation. This leads us to search certain new parameters which reflect the pathophysiology of ACS. Markers of plaque stability like Pregnancy Associated Plasma Protein-A and D-Dimer, a marker of ongoing thrombosis are found to be better markers in early diagnosis.

Aim: To evaluate the diagnostic competence of PAPP-A and D-Dimer in acute coronary syndrome over CK-MB and to compare with the inflammatory marker High Sensitive C-Reactive Protein (hs-CRP) which is associated with atherosclerosis.

Materials and Methods: Fifty patients presenting with acute onset of chest pain to Emergency Department with or without ECG changes served as cases and 50 healthy people served as controls. Serum PAPP-A is measured by Enzyme Linked Immunosorbent Assay (ELISA), D-Dimer and hs-CRP by using Latex Turbidimetry method.

Results: A statistical significant difference of PAPP-A and D-Dimer was noted between the ACS and controls ($p < 0.001$) whereas CK-MB shows no much difference (p 0.09). Statistically significant positive correlation is noted between parameters.

Conclusion: PAPP-A marker of plaque instability and D-Dimer marker of ongoing thrombosis are raised in acute coronary syndrome and thus can be considered as one of the marker in ACS for diagnosis.

Keywords: NSTEMI, STEMI, UA

INTRODUCTION

With the change in lifestyle habits and increased incidence of atherosclerosis the incidence of acute coronary syndrome increased comprising the major group of presentation to the emergency department. Acute Coronary Syndrome (ACS) is a group of disorders consisting of Unstable Angina (UA), Non ST Elevated Myocardial Infarction (NSTEMI), ST Elevated myocardial infarction (STEMI) [1,2].

Among the various groups of ACS, NSTEMI and UA diagnosis is difficult with the routinely done investigations as ECG changes are not seen in both and the raise of cardiac markers which is a differentiating feature between them occurs lately. This is the cause of mislead or missing of diagnosis and delay in initiation of treatment [3].

The cardiac markers used widely at present are markers of cardiac ischemia which include CK-MB, Troponins, and Myoglobin. Minimum time taken for their expression in blood is 2 hours which delays the treatment [4]. In order to initiate treatment at early we need parameters that reflect the ongoing pathology i.e., atherosclerotic formation and rupture. Pregnancy Associated Plasma Protein-A (PAPP-A) secreted from fibroblasts and vascular endothelial cells enhances accumulation of inflammatory cells and lipid laden macrophages thus causing progression of atherosclerosis. PAPP-A also found to play role in atheroma rupture initiating the thrombosis [5]. Ongoing thrombosis in coronary vessels consumes platelets and clotting factors. This process being disorganized there is both fibrin deposition and degradation and thus fibrin degradation marker D-Dimer found to be increased in ACS [6].

With this view we aimed to study the changes of PAPP-A and D-Dimer in both cases and control subjects thus evaluating their diagnostic efficacy over the routinely done cardiac marker CK-MB in early period.

MATERIALS AND METHODS

Study was conducted in Department of Biochemistry, JJM Medical College after the approval of ethical committee for over a period of one year i.e., from September 2013 to August 2014.

All patients presented with chest pain to the emergency department of Bapuji Hospital & Chigateri General Hospital were taken as study subjects. After informed consent blood samples were collected by venipuncture under aseptic precautions at the time of admission with a precaution of considering the study subjects who presented within 2 hours of acute onset of chest pain, and both serum and plasma are separated and stored at -20^{0}c until the assay is done.

The study subjects with liver and kidney disorders, inflammatory arthritis, infections, brain ischaemia, tumours, bleeding disorders, DM, pregnant women and samples collected after 2 hours of acute onset of chest pain are excluded from the study group.

After exclusions based upon the diagnosis at the time of discharge by electrocardiogram and cardiac troponin 50 patients were grouped under cases i.e., ACS which include 35 STEMI, 15 NSTEMI cases and 50 apparently age and sex matched healthy individuals as controls.

PAPP-A was analysed by ELISA method as per the instructions given by the manufacturer DRG international, Germany [7]. Whereas hs-CRP& D-Dimer measured by Latex Turbidimetric method as per the instructions given by the manufacturer EURO Diagnostics & Tulip Diagnostics Chennai [8,9].

Serum levels of PAPP-A up to 2.5mIU/L, plasma D-Dimer up to 250ng/ml DDU, hs-CRP up to 3mg/L are considered as normal [10-12].

STATISTICAL ANALYSIS

All values are expressed in terms of Mean±SD and subjected to proper statistical analyses using Microsoft excel with QI macros software where Z-test applied to find out the statistical significance

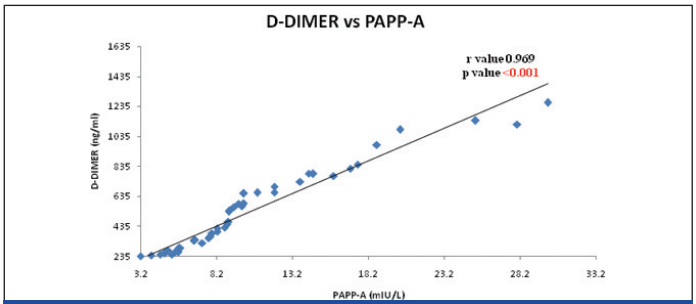
between cases & controls. Pearson correlation analysis was done for identifying the correlation between the parameters. P-value < 0.05 considered as statistically significant.

RESULTS

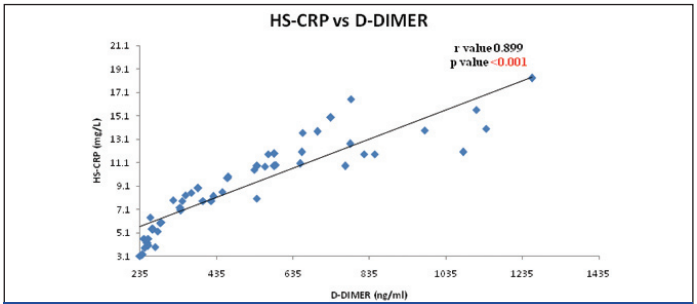
Mean values of PAPP-A, D-Dimer, hs-CRP were showing significant elevation in ACS over controls with a p-value of <0.001 whereas CK-MB values are not raised significantly in ACS as shown in the [Table/Fig-1]. This is because samples are collected within 2 hours of onset of chest pain and normally CK-MB rises after 4-6 hours of onset of symptoms reaching a peak after 24 hours. Pearson correlation analysis was done between the parameters a significant positive correlation is noted between them with a p-value of <0.001 as shown in [Table/Fig-2-4]. Among them a very strong positive correlation noted between PAPP-A and D-dimer with an r-value of 0.969.

Parameter	Controls	ACS	Z score	p-value
PAPP-A	1.2 ± 0.6	10.2 ± 5.9	10.54	<0.001
D-Dimer	29.5 ± 18.6	524.9 ± 266.4	12.9	<0.001
HS-CRP	1.5 ± 0.8	9.2 ± 3.7	14.26	<0.001
CK-MB	13.7 ± 4	14.9 ± 3	1.69	0.09

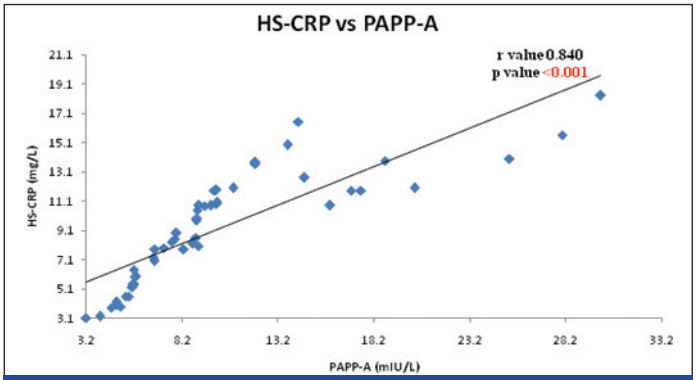
[Table/Fig-1]: Mean±SD of various parameters in ACS and Controls
PAPP-A: Pregnancy Associated Plasma protein-A HS-CRP: High Sensitive C-Reactive Protein
CK-MB: Creatine Kinase MB ACS: Acute Coronary Syndrome



[Table/Fig-2]: Correlation graph showing positive correlation between PAPP-A and D-Dimer in ACS
PAPP-A: Pregnancy Associated Plasma protein-A ACS: Acute Coronary Syndrome



[Table/Fig-3]: Correlation graph showing positive correlation between HS-CRP and D-Dimer in ACS
HS-CRP: High Sensitive C-Reactive Protein ACS: Acute Coronary Syndrome



[Table/Fig-4]: Correlation graph showing positive correlation between PAPP-A and HS-CRP in ACS
PAPP-A: Pregnancy Associated Plasma protein-A HS-CRP: High Sensitive C-Reactive Protein
ACS: Acute Coronary Syndrome

DISCUSSION

Pregnancy associated plasma/placental protein-A (PAPP-A) is a zinc metalloproteinase identified in 1973 for the first time [13]. It was introduced as marker of downs syndrome and used in prenatal diagnosis along with ultrasound Doppler and free β-HCG [14]. Later found to be useful in diagnosis of pre-eclampsia in early trimester [15]. PAPP-A initially thought to be secreted by placental syncitiotrophoblasts was found to be expressed in ACS in the 2001 [16].

Role of PAPP-A in pathogenesis of ACS was established when Beaudeux JL et al., found PAPP-A in echogenic carotid atherosclerotic plaques of non-symptomatic hyperlipidemic patients by immunohistochemistry thus proving PAPP-A plays a key role in atheroma formation [17]. Whereas Brugger-Anderson et al., had shown increased expression of PAPP-A in atherothrombotic plaques by immunohistochemistry techniques from STEMI patients during postmortem denoting its role not only in atheroma formation but has a role in plaque rupture [18].

PAPP-A via IGF-1 action enhances the atheroma formation by modification of extra-cellular matrix. It is also responsible for instability by fibrous cap thinning [19]. Present study shows significant difference of PAPP-A between the ACS and control groups which is long with studies of Bayes-Genis et al., Iversen et al., [5,19].

PAPP-A, a matrix metalloproteinase exhibits Insulin Growth factor (IGF) cleaving action from its binding protein Insulin Growth Factor Binding Protein-4 (IGFBP 4). The free IGF plays a key role in proliferation of vascular smooth muscle and fibroblast thus altering the peri vascular matrix and enhancement of atheroma formation [20,21]. Apart from atheroma enhancement it also causes chemotaxis of monocytes and neutrophils that leads to increased production of inflammatory markers like hs-CRP [22,23]. Because of increased PAPP-A, the enhancing atheroma leads to thinning of cap which causes increased fibrinogen accumulation and fibrin degradation products [24]. The increased inflammatory mediators further enhance the secretion of metalloproteinases like PAPP-A thus acting like a vicious cycle [25].

D-dimer a marker of cross linked fibrin turnover has been shown to be associated with risk of developing Ischemic Heart Disease (IHD) in future in people with & without baseline vascular disease [26-34]. Salomaa V et al., concluded that higher levels of D-Dimer seen in patients than controls suggesting the high turnover of fibrin, that fits the hypothesis of hyper-coagulable state which precedes the clinical CHD events [35]. In the present study we found significant high levels of D-dimer in ACS than in controls.

Arthur JM et al., found that higher levels of D-Dimer which reflects procoagulant state that contributes independently to recurrent coronary events [36]. Gordon et al., suggested that measurement of both D-dimer and CRP helps in enhancement of risk stratification for IHD [37].

High Sensitive CRP is a marker of plaque instability and inflammation where the levels are raised and noted at far early stages of atheroma formation Suleiman M et al., shown that CRP levels can predict the heart failure after episode of MI [38]. Raise of hs-CRP denotes the effect of inflammation secondary to myocardial necrosis in MI. Significant raise in hs-CRP-value is noted in acute coronary syndrome when compared with controls.

Studies also shown that PAPP-A and D-Dimer raised far early than the CK-MB currently used cardiac marker for ACS as both of them denotes ongoing pathogenesis whereas CK-MB reflects the effect of pathogenesis i.e., myocardial ischaemia [24]. A significant positive correlation is seen between the parameters denoting all are related to unstable athero thrombotic plaques and yet they differ with each other in predicting the outcome.

Heeschen et al., found that PAPP-A levels of >12.1 mIU/L denotes the risk of death/recurrent MI at 30 days & 6 months even with

negative troponin results. Though the present study doesn't comment regarding this because of lack of long term follow up of the study subjects [24].

Bayes Genis et al., found D-dimer level of >500 ng/ml had an independent diagnostic value for MI and increases the diagnostic sensitivity of ECG and history from 73% to 92% [39].

LIMITATION

Limitations of the present study are sample size not calculated and comparison with high sensitive cardiac troponin should have been done.

CONCLUSION

We found the markers of plaque instability like PAPP-A and markers of ongoing thrombotic activity like D-Dimer helps in early diagnosis than the routinely done cardiac ischemic markers in ACS. As they reflect the pathological process of ACS i.e., ongoing atherothrombosis PAPP-A and D-Dimer can be used to rule out the false positive cases like GERD and other non-cardiac chest pain that mimics cardiac chest pain. This help us in initiation of treatment appropriately thus decreasing the work load in emergency department.

Conflict of Interest: The authors declare no conflict of interest.

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