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Original Research Article

Platelet aggregation, mean platelet volume and plasma fibrinogen as risk factors for acute myocardial infarction

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ABSTRACT

Background: The Aim of this study was to assess the role of platelet aggregation, mean platelet volume (MPV) and plasma fibrinogen levels in the pathogenesis of acute myocardial infarction (AMI).
Methods: A prospective case control study was conducted on 30 cases of AMI and 30 normal healthy age and sex matched controls. The cases and controls were investigated for platelet aggregation studies (done in platelet rich plasma (PRP) using light transmission chrono-log optical aggregometer), MPV (measured by automated cell counter) and plasma fibrinogen levels (estimated by Clauss method).
Results: The mean platelet aggregation (%) in cases AMI was 57.61±11.91 which was significantly higher compared with 35.00±10.40 for healthy controls ($p<0.001$). Using Receiver Operating Characteristic (ROC) analysis, most patients of AMI had a platelet aggregability of ≥49% on optical aggregometry (sensitivity = 83.3 % and specificity = 93.7%). The MPV (fL) in cases of AMI was 8.04±0.39 which was significantly larger when compared with 7.67±0.43 for controls ($p= 0.001$). The mean plasma fibrinogen concentration in cases of AMI was 383.1±48.3mg/dl which was significantly higher when compared with 271.33±57.7mg/dl for healthy controls ($p<0.001$).
Conclusions: Platelet hyperaggregability, elevated MPV and plasma fibrinogen levels are found in patients with AMI and contribute significantly to risk of developing coronary thrombosis. These variables should be considered as additional screening tools to identify individuals at increased risk of developing AMI.

Keywords: Acute myocardial infarction, Fibrinogen, Mean platelet volume, Platelet hyperaggregation

INTRODUCTION

Coronary artery disease the leading causes of death worldwide and atherosclerosis is by far the most frequent underlying cause of coronary artery disease.[1,2] Atherosclerosis is characterized by accumulation of lipid-laden foam cells in the intimal layer of the artery and the development of a fibrofatty plaque which gradually enlarges over time and encroaches on the lumen of the artery. Acute coronary syndromes e.g. acute myocardial infarction (AMI) occur due to acute thrombotic occlusion of an epicardial vessel which occurs because of sudden disruption of an atherosclerotic plaque exposed to high shear stress at the site of stenosis or arterial branching.[3,4]

Conventional risk factors-older age, male sex, diabetes, hypertension, dyslipidemia, and smoking as established by the Framingham heart study do not explain the entire risk of coronary artery disease. This has led to the search for other risk factors particularly hemostatic and

rheological factors which may be involved in the pathogenesis of atherosclerotic vascular diseases.[5-7]

Platelets participate not only in thrombus formation during atheromatous injury, but also in the initiation and progression of atherosclerotic plaques.[8] Recent studies suggest that platelet activation, and platelet hyper-reactivity are associated with acute coronary syndromes.[9] During the process of activation, platelets undergo changes in shape and increase in size and become enzymatically and metabolically more active.[10-12] Therefore, platelet size measured as mean platelet volume can be used as a marker of platelet activity and function.[13]

Plasma fibrinogen promotes thrombosis by interacting with GPIIb/IIIa receptors on platelets and thereby causes platelet aggregation.[14] Plasma concentration of fibrinogen also strongly correlates with plasma viscosity and increase in plasma viscosity is associated with decreased blood flow rate and increased thrombotic affinity. In recent studies AMI was found to be associated with increase in plasma fibrinogen concentration.[15]

In this study, we assessed the role of platelet aggregation, mean platelet volume and plasma fibrinogen levels in the pathogenesis of AMI.

METHODS

This study was a tertiary hospital based prospective study undertaken at the Haematology Laboratory, Department of Pathology, and Department of Cardiology, King George's Medical University (KGMU), Lucknow from August 2009 to August 2010. This study was undertaken on 30 cases of AMI and 30 controls. The cases included in this study included 30 Patients who were admitted to Department of Cardiology, KGMU, Lucknow, and were diagnosed as cases of AMI based on their clinical history, examination, electrocardiography (ECG) findings. Patients receiving drugs interfering with platelet functions were excluded.

The controls included in this study were normal healthy individuals who registered for voluntary blood donation at Department of Pathology, KGMU, Lucknow. Only those blood donors who were not on any such medications which might have interfered with coagulation reaction or platelet function and who had no history of smoking, diabetes or hypertension were included as controls. The controls were age and sex matched with respect to the cases. Informed consent was obtained from both cases and controls. Peripheral venous blood samples were obtained from controls at the time of blood donation and from patients at the time of admission before any treatment could be initiated. MPV was assessed by an automated cell counter.

For platelet aggregation studies, 10ml venous blood was added to 3.8% trisodium citrate in 9:1 ratio contained in plastic tube. Platelet rich plasma (PRP) was obtained by centrifugation at room temperature (20-25^{0}C) for 5min at 1000rpm. PRP was removed carefully. Platelet Poor Plasma (PPP) was obtained by centrifuging the remaining blood (after separation of PRP) for 10min at 2000rpm. Standardization of PRP was done by measuring the optical density of PRP at 630nm and adjusting it between 0.75 to 0.80 which corresponds to a cell count of 2x10^8 cells/ml. For very high PRP count, the count was adjusted by diluting the PRP in the patient's PPP. Platelet count of less than 2x10^{8}cells/ml gives rise to diminished aggregation responses. The control PRP was also diluted to the same count and tested as a comparison. 5µM/ml working solution of adenosine 5-diphosphate (ADP) previously stocked at -80^{0}C was used as agonist. The chrono-log optical aggregometer (chrono-log corporation, Havertown, PA, USA) was switched on about 30min before the tests to be performed to allow the heating block to warm up to 37^{0}C. 500µl of PRP was pipetted into a cuvette which was placed in to the heating block for incubation. PRP was warmed up to 37^{0}C for 2min and then 5µL ADP was added. Change in absorbance was noted until the response reached a plateau or for 5min.

Plasma fibrinogen levels were estimated by the Clauss method. Plasma was obtained by centrifugation of citrated blood sample of patients and controls. A 1:10 dilution of plasma specimen with Owren's buffer solution was prepared. 0.2ml of 1:10 dilution of plasma sample was added to a test tube which was incubated at 37^{0}C for 1minute. Then 0.1ml of thrombin reagent (pre-warmed at 37^{0}C for 1min) was added and the stopwatch was started. The stopwatch was stopped at the first appearance of the fibrin web and the time in seconds was recorded in seconds. The test was repeated in duplication. The fibrinogen level was directly read off from the graph if the clotting time was between 5 to 50 seconds.

Statistical analyses of data obtained was done using Statistical Package for Social Sciences, version 23 (SPSS-23, IBM, Chicago, USA). Normality of the continuous data was tested. Continuous variables were presented in mean ± standard deviation while categorical variables were presented in frequency and percentage. To compare the means between cases and controls, independent samples t test was used. To find out the cut-off values of platelet aggregation to diagnose cases with corresponding diagnostic accuracy (including area under curve (AUC), sensitivity and specificity), receiver operating characteristic (ROC) curve was used. P value <0.05 was considered as statistically significant.

RESULTS

In this study, AMI was found to be commoner in males (73.3% cases; n=22) as compared to females (26.67% cases; n=8). The maximal incidence of AMI was in the 55 to 74-year age group (16 out of 30 cases; 53.33%).

The mean platelet aggregation (%) in male (n=22) and female (n=8) cases of AMI was 53.49±10.73 and

68.93±6.49 respectively which was significantly increased when compared with 31.88±10.45 for male controls (n = 22; t=6.765, p<0.001) and 43.58±2.40 for female controls (n = 8; t=10.356, p<0.001) respectively (Figure 1, Figure 2).

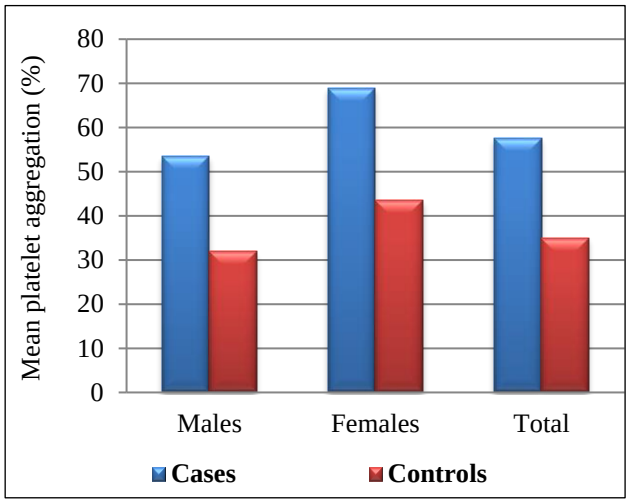
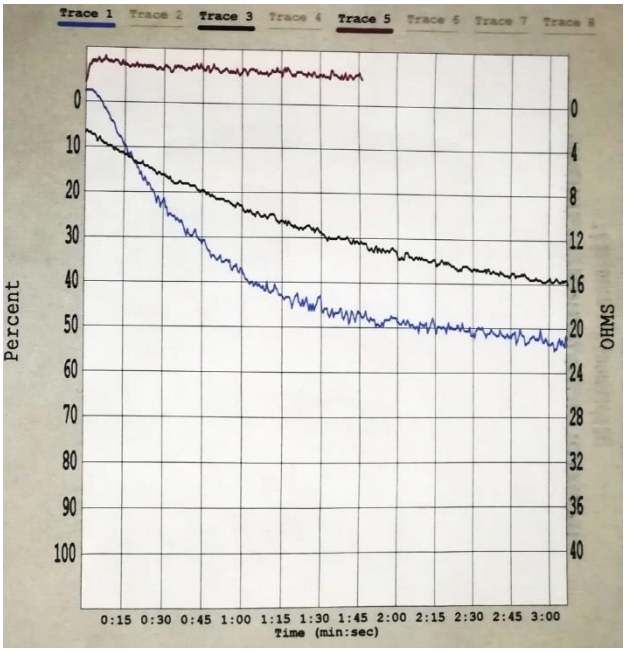


Figure 1: Mean platelet aggregation (%) in cases and controls.



Platelet hyperaggregation is seen in a patient of acute myocardial infarction (blue tracing showing approximately 56% platelet aggregation) as compared to control sample (black tracing showing approximately 37% platelet aggregation)

Figure 2: ADP induced platelet aggregation response by chrono-log optical aggregometer.

The mean platelet aggregation (%) in all cases of AMI (n=30) was 57.61±11.91 which was significantly higher as compared with 35.00±10.40 for healthy controls (n = 30; t=7.830, p<0.001) (Figure 1). The diagnostic accuracy of platelet aggregation calculated as the area under the curve by the receiver operating characteristic analysis was 0.926. At 41.5% aggregation cut off, the sensitivity and specificity of platelet aggregation for predicting AMI were 90% and 70% respectively. Similarly, the sensitivity and specificity of platelet aggregation for predicting AMI were 83.3% and 96.7% (at cut off values of 49%) and 80% and 100% (at cut off values of 50.5%) respectively (Figure 3, Figure 4).

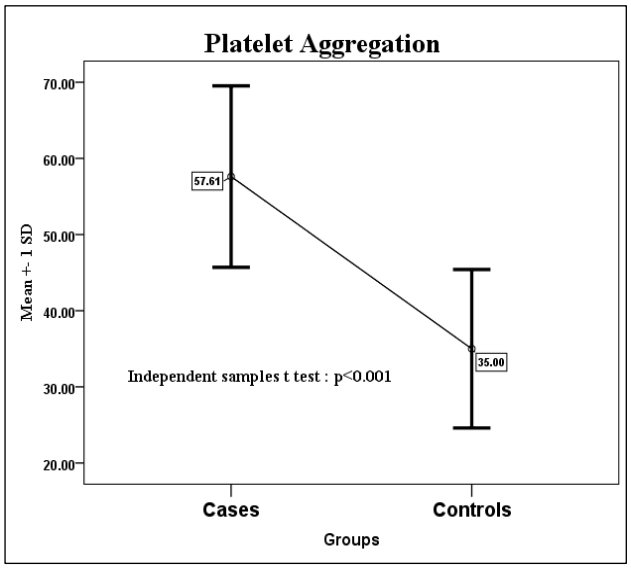


Figure 3: Mean±SD of platelet aggregability of cases and controls.

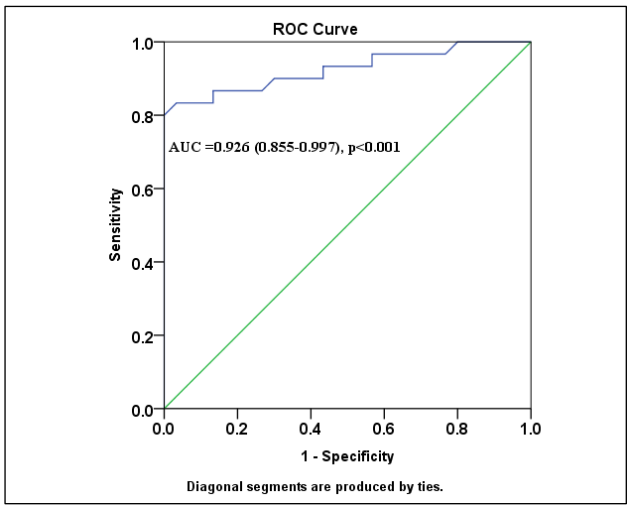


Figure 4: ROC curve showing area under curve (AUC) of platelet aggregability to diagnose AMI.

The MPV (fL) in all cases of AMI (n=30) was 8.04±0.39 which was significantly larger when compared with 7.67±0.43 for all controls (n = 22; t= 3.483, p= 0.001) (Figure 5).

The mean plasma fibrinogen concentration in cases of AMI (n=30) was 383.1±48.3 which was significantly higher when compared with 271.33±57.7mg/dl for all

healthy controls in this study (n = 30; t=16.814, p<0.001). The mean plasma fibrinogen concentration in male (n=22) and female (n=8) cases of AMI was 375.27±45.7mg/dl and 405.37±51.6mg/dl respectively which was significantly higher when compared with 262.27±55.2mg/dl for healthy male controls (n = 22; t=7.372, p<0.001) and 296.25±60.7mg/dl for healthy female controls (n = 8; t= 3.873, p =0.002) respectively (Figure 6).

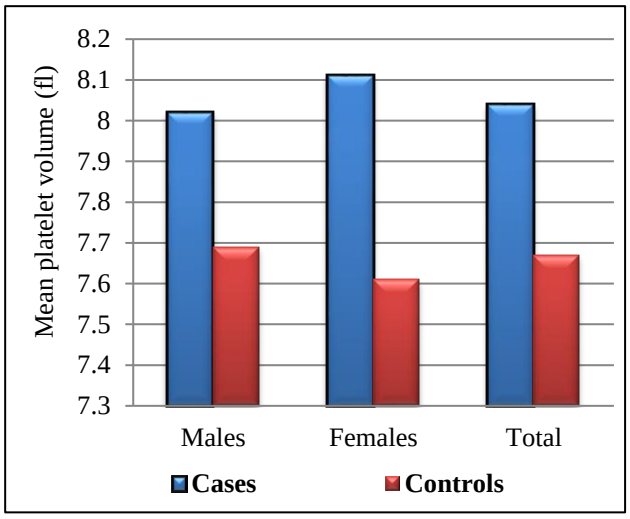


Figure 5: Mean platelet volume (fL) in cases and controls.

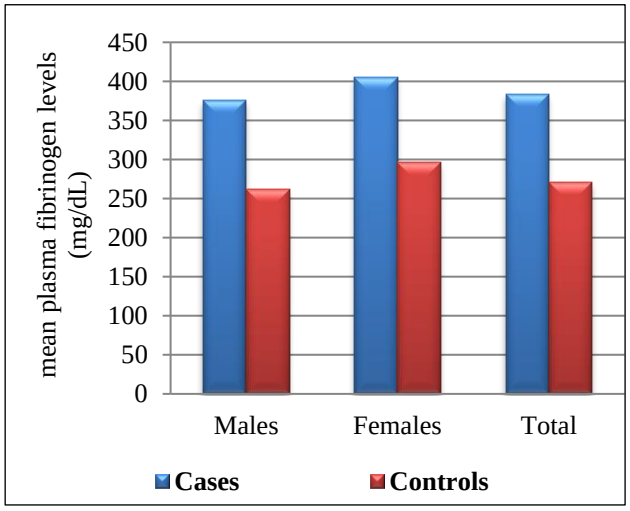


Figure 6: Mean plasma fibrinogen levels in cases and controls.

DISCUSSION

Rupture of atherosclerotic plaque exposes circulating platelets to Von Willebrand Factor (VWF) and sub endothelial collagen. VWF and collagen bind to platelet receptor GP Ib-V-IX and GP VI respectively.[16,17] This causes activation of GP IIb/IIIa receptor on the platelet surface and release of Thromboxane A_2 (TxA_2) and ADP from platelets. ADP causes recruitment and activation of resting platelets from the circulation to the site of plaque. TxA_2 causes local vasoconstriction and favours thrombosis by slowing blood flow.[17] Circulating fibrinogen binds activated GP IIb/IIIa receptors of activated platelets which leads to formation of platelet microaggregates which may embolize distally or progress to acute thrombotic occlusion of the epicardial vessel.[18]

In our study, we observed that platelets of patients with AMI were hyperaggregable when compared to platelets of controls on platelet aggregation testing with ADP as agonist on optical aggregometry. The mean platelet aggregation (%) in cases of AMI (n=30) was 57.61±11.91 (range=29-76) which was significantly increased compared with 35.00±10.40 (range=12-50) for healthy controls (n = 30) (p <0.001). Using Receiver Operating Characteristic (ROC) analysis, we observed that most patients of AMI had a platelet aggregability of ≥49% on optical aggregometry (sensitivity = 83.3% and specificity = 93.7 %).

Fuchs et al, in their study, found reversible platelet microaggregates under light microscopy on Giemsa stained blood smears from venous blood specimens of patients of stable angina (15±4%), unstable angina (24± 13%) and AMI (24±10%) which were significantly higher than that in normal healthy controls (6±2%).[19] It has been observed under shear stress based aggregometry, that platelets are hyperaggregable in conditions of increased shear stress even without stimulation by an agonist.[20] Eto et al, observed that patients with unstable angina showed tendency towards increased platelet aggregability under low shear even before the onset of myocardial infarction without the need of an agonist. They suggested that the GP IIb/IIIa receptor on platelets is already activated before the occurrence of coronary thrombosis in patients who are diagnosed as unstable angina.[21] Guha S et al, observed a high degree of platelet aggregation in the early hours after AMI which even though reduced after 48hours of standard antiplatelet remained significantly high. The above studies suggest that platelets are hyperaggregable in patients at high risk for developing AMI.[22] Therefore, we suggest that platelet aggregation testing can be used as an additional tool to identify persons at risk of developing AMI.

We observed significantly higher MPV in our cases of AMI as compared to controls. How increased platelet volume causes increased risk of AMI is not well understood. Chronic hypoxia has been shown to stimulate cytokine (interleukin 3, interleukin 6, thrombopoietin) mediated production of larger platelets from the bone marrow. Larger platelets have been found to be metabolically and enzymatically more active. They express higher levels of procoagulatory surface proteins (P-selectin, glycoprotein IIIa) and therefore play a crucial role in prothrombotic events leading to AMI after rupture of an atherosclerotic plaque.[23] A graded, independent association between baseline MPV level and risk of

cardiovascular mortality, stroke, fatal or non-fatal MI and cardiac failure at 1 year was observed in a study by Ranjith et al, suggesting that the time span between AMI and laboratory testing does not influence the MPV. i.e. MPV does not change during an episode of AMI rather it is an important predisposing factor for AMI.[24]

Fibrinogen is involved in atherothrombotic processes by multiple mechanisms. Fibrinogen is an acute phase reactant whose levels are increased in acute coronary syndromes.[25] It promotes endothelial cell migration and extracellular low-density lipoprotein accumulation. Fibrinogen interacts with GPIIb/IIIa receptors on platelets to promote platelet aggregation. An elevated plasma fibrinogen level increases blood viscosity, which causes impaired microcirculatory flow, endothelial shear-stress damage, and predisposition to thrombosis.[26] This study showed that patients with AMI had significantly higher fibrinogen levels than controls. The mean plasma fibrinogen concentration in cases of AMI (n=30) was 383.1±48.3 which was significantly higher when compared with 271.33±57.7mg/dl for all healthy controls in this study (n = 30; p<0.001).

CONCLUSION

In conclusion, Platelets and plasma fibrinogen play an important role in the atherothrombotic processes that lead to AMI. They observed that patients of AMI have hyper-aggregable platelets, elevated MPV and increased plasma fibrinogen levels. Therefore, we suggest that platelet aggregation testing and testing for MPV and plasma fibrinogen levels may be used as additional tools to detect patients at high risk of AMI.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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