

## Levels of platelet-derived microparticles and soluble p-selectin in patients of acute myocardial infarction (case control study)

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### Abstract

**Objective:** To measure levels of platelet-derived microparticles and soluble P-selectin in patients of acute myocardial infarction and their comparison with healthy controls.

**Methods:** This case-control study was conducted in Department of Haematology, University of Health Sciences Lahore from April to September 2013, and comprised patients of acute myocardial infarction in group 1 and healthy controls in group 2. Platelet-derived microparticles and soluble P-selectin were measured by enzyme-linked immunosorbent assay. SPSS21 was used for data analysis.

**Results:** Of the 80 participants, 50(62.5%) were patients and 30(37.5%) were controls. The mean levels of platelet-derived microparticles and soluble P-selectin were significantly higher in group 1 compared to group 2 ( $45.70 \pm 10.30$  vs  $10.60 \pm 0.96$ , and  $51.46 \pm 9.30$  vs  $9.16 \pm 1.04$ , respectively) ( $p < 0.001$ ). There was no significant difference in levels of platelet-derived microparticles and soluble P-selectin in three intervals after acute myocardial infarction ( $p > 0.05$ ). Although levels of platelet-derived microparticles and soluble P-selectin did not correlate to creatinekinase-myocardial band levels ( $p > 0.05$ ), but there was a trend of significant correlation with cardiac troponin T ( $p < 0.05$ ).

**Conclusion:** Levels of platelet-derived microparticles and soluble P-selectin can be used as novel early diagnostic marker of acute myocardial infarction.

**Keywords:** Platelet-derived microparticles, Soluble P-selectin, Acute myocardial infarction, Cardiac troponin T, Creatine-kinase MB. (JPMA 67: 998; 2017)

### Introduction

Myocardial infarction (MI) is a major cause of death and disability worldwide. Platelets play a crucial role in the pathogenesis of acute myocardial infarction (AMI). Activated platelets adhere to the vessel wall at the site of a ruptured plaque and initiate formation of an arterial thrombus, which leads to infarction.<sup>1</sup> This increased platelet activation is reflected in the elevation of platelet-derived microparticles (PDMPs) and soluble P (sP)-selectin.<sup>2</sup> Increasing evidence suggests that markers of platelet activation can be used to identify the disease activity.<sup>3</sup> Although AMI is the most common diagnosis occurring in hospitalised patients, it has been seen that 50% of patients hospitalised for suspected acute coronary syndrome ultimately leave the hospital with other diagnosis. Currently, cardiac biomarkers are an integral part for the diagnosis of AMI. Among all, cardiac troponin appears to be the most sensitive and specific biomarker, but it is insufficient because it will not detect 10 to 15% of patients at risk. Cardiac troponin and creatinekinase (CK)-

myocardial band (MB) also does not permit early detection of MI. So, there is a need of ideal biomarkers with high sensitivity and specificity for early diagnosis (1-3h) having prognostic and therapeutic implications.<sup>4</sup>

Microparticles (MPs) constitute a heterogeneous population, differing in cellular origin, numbers, size, antigenic composition, and functional properties. They are derived from various cells including platelets, leucocytes, lymphocytes, erythrocytes, and endothelial cells.<sup>5</sup> However, PDMPs represent approximately 70-90% of circulating microparticles in the cardiovascular system.<sup>6</sup> PDMPs are potentially procoagulant, since they expose negatively charged phospholipids, possess activated coagulation factors Va and Xa, as well as tissue factor. PDMPs have been demonstrated to be increased in patients with AMI, transient ischaemic attacks, cardiopulmonary bypass and peripheral arterial disease.<sup>2,7</sup>

P-selectin (CD62P) is a cell-adhesion molecule produced by activated platelets and endothelial cells. It is rapidly translocated from platelet  $\alpha$ - granules and endothelial weibel-palade bodies to the cell surface. Consequently, enzymatic cleavage of expressed p-selectin and alternative splicing of the messenger ribonucleic acid of p-selectin occur quickly, giving rise to sP-selectin in the peripheral blood that can be detected.<sup>8</sup> Soluble p-selectin

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is considered to be a marker of platelet activation and a direct inducer of pro-coagulant state.<sup>9</sup>

The current study was planned to measure levels of PDMPs and sP-selectin in AMI patients by enzyme-linked immunosorbent assay (ELISA) and compared them with healthy controls. Additionally, we analysed the correlation of PDMPs and sP-selectin with troponin T (TnT) and CK-MB. We also analysed serial changes in levels of PDMPs and sP-selectin after acute MI.

## Patients and Methods

This case-control study was conducted in Department of Haematology, University of Health Sciences Lahore from April 2013 to September 2013, and comprised AMI patients in group 1 and healthy controls in group 2. The study was approved by local ethics committee. Patients were enrolled after informed written consent. The sample size was calculated by keeping the power of study equal to 90% and level of significance equal to 5%.<sup>10</sup>

Desired power = 90%; desired level of significance = 5%; mean difference = 47.3 - 36.8 = 10.5; standard deviation of group 1 = 15; standard deviation of group 2 = 11.

The calculated sample size was 33 in each group, but it was increased for more statistical accuracy. Additionally, ELISA was used to measure the levels of PDMPs and soluble P-selectin in this study.

Patients with typical chest pain  $\geq 30$  minutes and  $\leq 12$  hours, ST segment elevation or depression in at least two contiguous leads on electrocardiogram (ECG), cardiac troponin +ve and CK-MB ( $> 24$  U/L) were included. By contrast, patients with any acute or chronic inflammatory disease, malignant disorder, hepatic or renal dysfunction, history of venous thromboembolism, stroke, anticoagulant therapy or previous coronary artery bypass surgery were excluded from the study. Patients' age- and sex-matched healthy controls were also selected. This matching was in a whole group, not in a pair-wise manner. Controls with any inflammatory disorder, malignancy, cardiovascular risk factors or on anticoagulant therapy were excluded. Controls were either spouses or acquaintances of patients, hospital staff or their friends.

Samples were taken before the start of anticoagulant or thrombolytic therapy. Five ml blood was collected with a 21-gauge needle to minimise platelet activation using an aseptic technique. It was divided into two vacutainers. Two ml was transferred in vacutainers containing 1/10 volume of ethylenediaminetetraacetic acid (EDTA)- acid-citrate-dextrose (ACD) anticoagulant for analysis of PDMPs. It was centrifuged at 4000g for 10 minutes to get

platelet-poor plasma at room temperature and 200 $\mu$ L from upper layer supernatant was collected and stored in 0.5ml Eppendorf tubes at  $-80^{\circ}\text{C}$  until analysis by Human PDMP ELISA kit (Glory Science Co. Ltd, United States) which is a quantitative sandwich immunoassay kit.

Then, 3ml blood was transferred in vacutainers containing sodium citrate for sP-selectin measurement and was centrifuged at 3,000 revolutions per minute for 20 minutes at room temperature to get platelet-poor plasma. This was stored in 0.5ml Eppendorf tubes at  $-80^{\circ}\text{C}$  until analysis by commercially available solid-phase sandwich ELISA kit (Glory Science Co. Ltd). All samples were processed within one hour of sample collection. All samples were thawed for 15 minutes at  $37^{\circ}\text{C}$  before analysis.

TnT (Elecsys 2010, Roche) and CK-MB (912 Automatic analyser, Roche) were measured according to the manufacturer's instructions. Cardiac TnT and CK-MB were measured at admission and then at 4-6 hours if initial results were negative. Platelet count, total cholesterol and random blood sugar measurements were made using standard laboratory methods.

Data was analysed using SPSS21. Continuous variables were expressed as mean  $\pm$  standard deviation (SD), whereas categorical variables in the form of frequency and percentage. Student's t-test was used to compare PDMPs and sP-selectin and other continuous variables in cases and controls. Student's t-test was applied because test variables were continuous and grouping variable was categorical, whereas logistic regression is best one when both test variables and grouping variable are categorical.

Comparisons were also made by using Student's t-test. Pearson's correlation coefficient was used for correlation analysis because it is used to determine relationship between continuous variables.  $P \leq 0.05$  was considered significant.

## Results

Of the 80 participants, 50(62.5%) were patients and 30(37.5%) were controls. Of the patients, 43(86%) had ST-segment elevation MI and 7(%) had non ST-segment elevation MI. There was no difference in patients and controls with respect to age ( $p=0.826$ ), gender ( $p=0.649$ ), diastolic blood pressure ( $p=0.24$ ) and platelet count ( $p=0.714$ ). However, there was significant difference in body mass index ( $p=0.001$ ), pulse ( $p=0.03$ ), systolic blood pressure ( $p=0.05$ ), total cholesterol ( $p=0.001$ ) and random blood sugar ( $p=0.01$ ). None of the controls manifested cardiovascular disease clinically and were also negative for risk factors of cardiovascular disease. Mean value of

**Table-1:** Demographic characteristics of study population.

Variable	Patient (n = 50)	Controls (n = 30)	P-value
Males	42 (84%)	24 (80%)	0.649
Females	8 (16%)	6 (20%)	0.649
Age (yrs)	54.36±10.22	53.8±12.18	0.826
Pulse (/min)	84.74±14.62	78.56±5.80	0.03*
BMI (kg/m <sup>2</sup> )	25.51±2.25	23.12±0.86	<0.001*
BP (diastolic)	79.0±13.13	76.0±6.21	0.24
BP (systolic)	124.4±26.43	114.5±9.50	0.05*
Total cholesterol	200.4±36.7	142±28.75	<0.001*
RBS(mg/dl)	168.8±87.5	126 ± 21.34	0.01*
Platelets(10 <sup>9</sup> /L)	253.18±64.57	258.73±66.95	0.714
CK-MB(U/L)	77.12±54.30	--	--
TnT(pg/ml)	748.71±933.8	--	--

Data is given in mean ± SD or in n(%).

BMI: Body mass index

BP: Blood pressure

CK-MB: Creatine kinase-myocardial band

TnT: Troponin T

RBS: Random blood sugar

SD: Standard deviation

No: Number

\*p value ≤ 0.05.

**Table-2:** Clinical characteristics of patients.

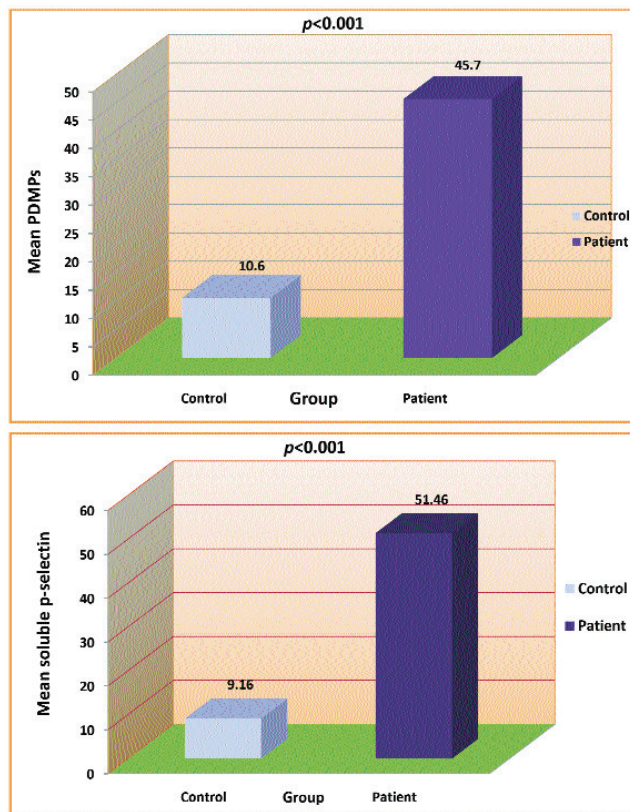
Variables	Frequency	Percentage	
Smoking	Current smokers	15	30%
	Ex-smokers	7	14%
	Non-smokers	28	56%
Hypertension (n = 19)	No medication	6	12%
	Antihypertensive drugs	13	26%
Diabetes mellitus (n = 20)	No medication	11	22%
	Oral hypoglycaemic	5	10%
	Insulin injection	4	8%
Hypercholesterolaemia (n = 16)	No medication	12	24%
	On statin	4	8%
H/o Angina	-	6	12%
Family H/o IHD	-	21	42%
Aspirin therapy	-	12	24%

Data is given in No. and %.

IHD: Ischaemic heart disease.

troponin T was 748.71±933.8 pg/ml (range: 106.8-5100 pg/ml) while mean value of CK-MB was 77.12±54.30U/L (range:27-301U/L) (Table-1).

Mean duration of chest pain was 5.64±3.66 hours (range: 1-12hours). Of all the patients, 15(30%) presented within 3 hours of chest pain, 22(44%) within 6 hours while 13(26%) presented within 12 hours of chest pain. Moreover, 15(30%) patients were smokers, 19(38%) had hypertension, 20(40%) had diabetes mellitus, 16(32%)



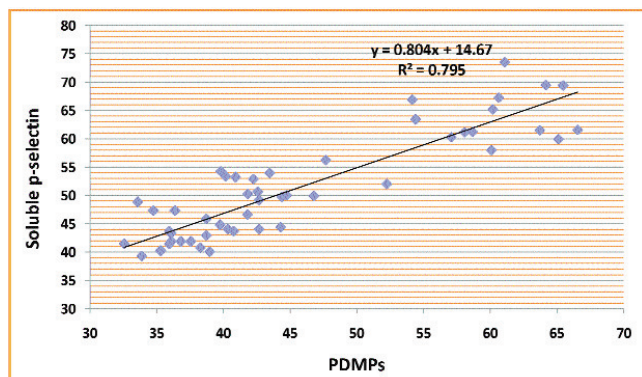
PDMPs: Platelet-derived microparticles  
sP-selectin: Soluble P-selectin

**Figure-1:** Mean levels of PDMPs and sP-selectin in controls and patients. Bar charts underline the significant increase in levels of PDMPs and sP-selectin in patients as compared to controls. Dark bars represent patients while lighter bars represent controls.

had hypercholesterolaemia, 6(12%) had history of angina and 21(42%) had family history of ischaemic heart disease (IHD) (Table-2).

Plasma levels of PDMPs and sP-selectin were significantly higher in patients of AMI than controls (45.70±10.30 vs 10.60±0.96 and 51.46±9.30 vs 9.16 ± 1.04, respectively) (p<0.001) (Figure-1).

PDMPs and sP-selectin levels were not influenced by age, gender, smoking status, arterial hypertension or hyperlipidaemia. Additionally, there was no statistically significant difference in levels of PDMPs and sP-selectin in patients with or without aspirin therapy (p=0.213 and p=0.852, respectively). However, diabetic patients had increased levels of PDMPs and sP-selectin (p=0.002 for PDMPs and p=0.01 for sP-selectin). Analysis description for categorical variable comparison has been described in detail in thesis but not in article coz of limitation of number of tables.



PDMPs: Platelet-derived microparticles  
 sP-selectin: Soluble P-selectin  
 AMI: Acute myocardial infarction

**Figure-2:** Scatter plot between plasma levels of PDMPs and sP-selectin. X-axis represents plasma levels of PDMPs while Y-axis shows plasma levels of soluble P-selectin in patients of AMI.

One-way analysis of variance (ANOVA) demonstrated an absence of serial changes in plasma levels of PDMPs and sP-selectin ( $42.83 \pm 8.38$ ,  $45.51 \pm 10.16$ ,  $49.34 \pm 12.08$  and  $48.73 \pm 7.45$ ,  $51.27 \pm 9.28$ ,  $54.93 \pm 10.72$ , respectively) in three intervals from the start of chest pain to blood sample collection  $\leq 3$  hours,  $> 3 \leq 6$  hours and  $> 6 \leq 12$  hours, respectively, following AMI. Although there was evidence of an increasing trend in plasma levels of PDMPs and sP-selectin with time, this trend was not significant ( $p=0.252$  and  $p=0.215$ , respectively).

Pearson's correlation demonstrated that there was positive and statistically significant correlation between plasma levels of PDMPs and sP-selectin in patients of AMI ( $r=0.892$ ,  $p < 0.001$ ).

Levels of PDMPs and sP-selectin did not correlate to platelet count, CK-MB and TnT, but there was a tendency of significant correlation with TnT (Figure-2).

## Discussion

Platelet activation results in formation of PDMPs and soluble p-selectin. The elevation of these platelet activation markers in acute myocardial infarction has been suggested in literature years ago. However, there appears to be no available data in Pakistan regarding circulating levels of PDMPs and sP-selectin in patients of AMI. To our knowledge, this study was the first regarding these markers. The main aim of our study was to measure plasma levels of PDMPs and soluble p-selectin in patients of acute myocardial infarction and their comparison with controls.

In our study, we clearly demonstrated that levels of both

PDMPs and soluble p-selectin were increased in patients of myocardial infarction as compared to controls ( $p < 0.001$ ). Our results are consistent with a previous study conducted by EwaStepien et al.<sup>3</sup> They specifically observed that tissue-factor bearing PDMPs (CD42/CD142) were increased in patients of AMI, suggesting procoagulant state in AMI. This is of pathophysiological and diagnostic interest because PDMPs are procoagulant and increase thrombin generation and fibrin formation adding to the body of knowledge that their levels reflect the extent of ischaemic and thrombotic burden.<sup>11</sup> In the current study, levels of PDMPs in patients with AMI  $< 3$  hours were significantly higher than that in controls and also there was no statistically significant difference in levels of PDMPs in three intervals after AMI.<sup>12</sup> Moreover, as plaque rupture and platelet activation occur before myocardial ischaemia and necrosis. Collectively, based on these findings, PDMPs can be proposed to be early diagnostic markers of AMI.

In our study, we observed that elevation of circulating PDMPs correlates with sP-selectin. This is in agreement with other studies.<sup>3,13</sup> These findings support the definitive role of PDMPs and soluble p-selectin in progression of atherosclerosis as well as thrombosis.

We could not find correlation between PDMPs and platelet count. This favours the systemically increased platelet reactivity in patients of AMI.<sup>14</sup> Furthermore, it supports the view that PDMPs are markers of ongoing thrombosis as elevated levels in MI are related to thrombus score.<sup>15</sup> On the contrary, it has been previously suggested that platelet count is a significant factor for predicting plasma PDMPs levels, and elevated PDMPs are associated with the 10-year coronary heart disease risk score in healthy men.<sup>16</sup> The possible reason for this might be the fact that their study included healthy men without signs, symptoms or history of cardiovascular disease, whereas our study comprised patients of acute MI who definitely had different platelet response. They might have increased platelet hyper-reactivity (defined as residual platelet activity despite anti-platelet drug therapy).<sup>17</sup>

Regarding correlation of PDMPs and sP-selectin with troponin T and CK-MB levels, our results differ from those observed by Jung et al.,<sup>7</sup> who observed that PDMPs correlated to the area under the cover of troponin T levels (AUC TnT), but not to CK-MB. In the current study, levels of PDMPs and sP-selectin had tendency towards significant correlation with troponin T. The lack of correlation between PDMPs and TnT might be due to the fact that we used levels of troponin T and CK-MB at admission, not area under the curve levels nor peak levels. Secondly,

troponin and CK-MB are released from necrotic myocardium, so reflecting infarct size while PDMPs and sP-selectin reflect platelet activation. Additionally, the severity of an MI depends on three factors: the level of occlusion in the coronary artery, the rate and the duration of the occlusion, and the extent of collateral circulation. The ischaemic injury may also depend on other factors, including age and comorbidities (hypertension, hyperlipidaemia, diabetes mellitus).<sup>18</sup>

In this context, it is interesting to note that a previous study demonstrated that despite being involved in the initiation of the atherothrombotic cascade, PDMPs do not reflect severity of myocardial damage. Indeed, PDMPs have been reported to be independent predictors of thrombotic events as these platelet activation markers and troponin reflect different aspects of pathophysiology of MI.<sup>12,19,20</sup> Similarly, a recent follow-up study demonstrated that soluble P-selectin has prognostic value in predicting the cardiac events better than troponins.<sup>21</sup>

In the present study, we also observed increased levels of sP-selectin in patients of acute myocardial infarction in comparison with controls. A growing number of studies have reported this. Soluble P-selectin is a marker of platelet activation which remains persistently elevated after MI.<sup>22</sup> Furthermore, plasma levels of sP-selectin exhibited no significant serial changes within 12 hours of AMI. Accordingly, we suggest that sP-selectin is rapidly expressed and rapidly released from activated platelets into circulation following AMI. So, it may be utilised in the emergency room as an early diagnostic tool for AMI. Our findings confirm previous reports<sup>23,24</sup> and extend the findings of these studies by showing that sP-selectin has an early diagnostic and prognostic value for AMI which is independent of cardiac troponin T and ECG findings. By combining initial troponin, ECG findings and p-selectin level at presentation, it was possible to achieve a sensitivity of 97.6% for AMI.<sup>25</sup>

Our study supported the accumulating evidence that PDMPs and sP-selectin are significantly higher in MI than in controls. Most importantly, no significant serial change in increased levels of PDMPs and sP-selectin was observed within 12 hours of AMI, raising the question whether PDMP and sP-selectin should be used as reliable and applicable biomarkers for early detection of AMI as already suggested by others.<sup>26</sup> In our opinion, however, these are not ready to be used as biomarker. Research on MPs currently faces several challenges in the area of standardisation of methods of detection as well as in standardisation of pre-analytical variables. So, further

studies are needed to elucidate their possible role as a diagnostic tool and possibly also as a therapeutic target.

Our study has some limitations, the main one being its small sample size. Furthermore, objective three was not prime objective of this study but was an additional finding and purpose of research is to explore new findings too. The rationale of studying the correlation between these novel markers of AMI with established markers like troponin and CK-MB was to analyse the efficacy of PDMPs and p-selectin as diagnostic markers of AMI. We might have observed different results regarding correlation analysis, if we had used peak levels of TnT and CK-MB. These are additional findings which were observed in this research and these highlight more need of research in this field.

## Conclusion

Levels of platelet-derived microparticles and soluble P-selectin can be used as novel early diagnostic marker of acute myocardial infarction.

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**Conflict of Interest:** None.

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