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Analysis of platelet parameters in patients with non-hematological disorders

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Abstract

***Background & Aims*:** People with diabetes, exhibit increased platelet reactivity. Both insulin resistance and insulin deficiency increase platelet reactivity. Platelets play a vital role in the pathogenesis of acute coronary syndromes (ACS). It has been shown that PC and MPV are independent predictors for poor outcome in primary intracerebral hemorrhage. The aim of the study is to analyze the platelet parameters in patients with some non-hematological disorders such as diabetes mellitus type 2, acute coronary syndrome, and acute ischemic stroke.

***Materials & Methods*:** Blood samples were collected from 400 subjects and analysed using the Sysmex KX-21 automated hematology analyser. 300 patients presenting with non-hematological disorders and 100 age and sex matched healthy controls were checked for Platelet indices such as PC, MPV, PDW and P-LCR. The platelet morphology was studied on peripheral blood smear for considering the arrangement, granularity and size of platelets.

***Results*:** In total non-hematological disorders, DM type 2 accounts 37.33% (n=112), followed by 35.67% of ACS (n=107), and 27% of AIS (n=81). In diabetes mellitus type 2, PC was statistically elevated along with MPV compared with healthy controls. MPV and PDW were significantly raised in the patients admitted with ACS. In acute ischemic stroke, we noted that there was an elevation in MPV and a decrease in PC compared to them in the control group. Study showed discrete arrangement of platelets in 90% of DM type 2, 88% of ACS, and 80% of AIS patients. PBS in various clinical conditions showed granularity of platelets in 84 % of DM type 2, 81% of ACS and 83% of AIS patients. Study showed increased percentage of macrothrombocytes as 60% of DM type 2, 56% of ACS and 52% of AIS patients. The percentage of large platelets on PBS was increased beyond normal limits in DM type 2, ACS and AIS cases, indicating that there is an increase in size of the platelets supporting an increase in MPV.

***Conclusion*:** The measurement of platelet indices may provide useful diagnostic and prognostic information to emergency physicians caring for patients with ACS and AIS.

***Keywords*:** Platelet Indices, Acute Coronary Syndrome, Diabetes Mellitus, Acute Ischemic Stroke

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Introduction

People with diabetes, exhibit increased platelet reactivity. Hyperglycemia contributes to greater platelet reactivity through direct effects and by promoting glycation of platelet proteins. Both insulin resistance and insulin deficiency increase platelet reactivity. Insulin inhibits activation of platelets. Therefore, relative or absolute deficiency of insulin would increase platelet reactivity (1).

Mean platelet volume (MPV) is an indicator of average size and activity of the platelets and is reported to be high in diabetes mellitus and is considered as a risk factor for heart disease. Platelet adhesion and aggregation are key functions leading to thrombus formation. The progression of atherosclerotic lesions seems to concur with increased thrombopoiesis activation where the cytoplasmic maturation of megakaryocytes is faster than the nuclear maturation, originating macroplatelets that produce more thromboxane A2 and show greater reactivity in platelet aggregation curves. The interaction of platelets with endothelial cells leads to an excessive platelet activation, which results in shorter half-life and increased platelet turnover. It may influence the platelet count (PLT), Mean Platelet Volume (MPV) as well as the Large Platelets percentage (LPLT) (2, 3). Within the atherosclerotic plaque, platelets could remain activated for a long time providing for proinflammatory cytokines production (4). Large platelets have also been reported in patients with high vascular risk factors and have also been associated with myocardial damage in acute coronary syndromes with an unfavorable outcome of acute myocardial infarction observed in survivors (5, 6).

Platelet Distribution Width (PDW) is an indicator of variation in platelet size which may be a sign of active platelet release. Platelet large cell ratio (P-LCR) is directly related to PDW and MPV (7).

Larger platelets usually contain more granules, thus releasing more chemokines promoting further platelet aggregation as well as activation. Elevated MPV simultaneously with the elevated PC increases the risk of thrombosis (8). Significant increased MPV in patients with deep vein thrombosis and isolated elevated PC in patients with pulmonary embolism has been revealed (9).

It has been shown that PC and MPV are independent predictors for poor outcome in Primary Intracerebral Hemorrhage (PICH) (10). Some reports reported that patients with acute stroke had significantly increased MPV or PC compared with controls (11), whereas other studies found decreased MPV or PC values in acute stroke patients (12).

Hence, keeping these aspects, we aimed to analyse the platelet parameters in patients presenting with non-hematological disorders such as diabetes mellitus type 2, acute coronary syndrome, and acute ischemic stroke. Study also analysed the platelet morphology on peripheral blood smear considering arrangement, granularity and size of platelets and comparing it with the control group.

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Materials & Methods

This is a prospective case-control study and data was collected for the duration of 25 months at Dr. Pinnamaneni Siddhartha Institute of Medical Sciences & Research Foundation (Dr. PSIMS & RF), Vijayawada, India. The study was conducted among 300 patients presenting with non-hematological disorders attending outpatient department or admitted in the hospital. A hundred age and sex matched controls were also collected. The institutional ethical committee approved the study protocol. Total 400 subjects were studied in two groups. Group 1 consisted of 300 patients with non-hematological disorders. In group 2, there were 100 healthy volunteers as the control group. Non-hematological disorders such as acute coronary syndrome, acute ischemic stroke and diabetes mellitus type 2 were studied.

Diabetes mellitus type 2: Patients diagnosed of diabetes mellitus type 2 with typical clinical manifestations (polyuria, polydipsia, weight loss) and laboratory tests (random plasma glucose >200mmg/dL) were included. Male patients with hemoglobin below 13 gm% and female patients below 12 gm% were excluded from the study because nutritional anemias

can be a cause for reactive thrombocytosis and hence, increased MPV. Patients with coronary artery disease and on antiplatelet drugs such as aspirin and clopidogrel were also excluded.

Acute coronary syndrome (ACS): Patients diagnosed of ACS with ECG changes (T-wave tenting or inversion, ST- segment elevation or depression and pathologic Q waves), laboratory tests (increased troponin T or I as well as CK-MB) were included. Patients with severe hepatic impairment, renal impairment or malignancy, and patients on anti-inflammatory, anti-coagulants or anti-platelet therapy were excluded.

Acute Ischemic Stroke (AIS): Patients diagnosed of AIS with typical history and clinical manifestations (awakening with or experiencing the abrupt onset of focal neurologic deficits) with brain computerized tomography showing ischemic stroke were included. Patients with infection, inflammatory disease, trauma, underlying hematological disease, autoimmune disease, malignancy as well as patients with clear source of emboli showering were excluded.

Controls: Controls were primarily hospital-based or who came for a routine check-up. Individuals with normal hemogram (Hb >12 gm%), normocytic normochromic peripheral blood smear, random blood glucose < 80 mg/dL and no known history of coronary artery disease or cerebrovascular accident were included. Individuals with neoplastic and chronic inflammatory disorders such as rheumatoid arthritis and systemic lupus erythematous as well as on anti-inflammatory, anti-coagulants or anti-platelet therapy were excluded.

Procedure: Venous blood samples were collected from the antecubital vein using a 5 cc syringe and transferred to vacutainers containing dipotassium EDTA. The hemograms were studied with special emphasis on platelet parameters specifically, PC, MPV, PDW, and P-LCR, and a peripheral smear was done. Platelet count was done on peripheral blood smear for all the hemograms showing platelet counts out of the normal range to rule out spurious causes of either thrombocytopenia or thrombocytosis by Fonio method (13). With a 1,000-fold magnification (eyepiece 10, lens 100), one platelet per field is equivalent to 20,000 platelets/μL in circulating blood. The platelet morphology was studied considering the arrangement, granularity, and size of platelets.

Statistical methods: The mean and standard deviation were calculated for different parameters in both the patient and control groups. The comparison of the means was done using students unpaired 't' test. Test of proportion was applied wherever necessary. In all above tests 'p' value less than 0.05 was taken to be statistically significant.

Results

Group 1 consisted of 300 patients with non-hematological disorders. Non-hematological disorders such as DM type 2, ACS, and AIS were studied. Group 2 consisted of 100 healthy volunteers as the control group.

Table 1. Characteristics of the control group

Characteristic	Control group
Number	100
Age (years)	39.86 ± 11.98
Male (%)	51 (51%)
Female (%)	49 (49%)
Hemoglobin (gm%)	14.2 ± 0.69
MCV (fL)	87.4 ± 4.6
MCHC (gm/dL)	32.5 ± 1.7
TLC (x μL)	6911.2 ± 1313.98
PC (x 10^9/L)	249.94 ± 56.38

MPV (fL)	9.5 ± 0.89
PDW (fL)	11.0 ± 1.14
P-LCR (%)	20.5 ± 2.99

On PBS, the platelet morphology of control group appeared as 93% discrete, 95% granular, and there was no increase in the percentage of large platelets. In non-hematological disorders, DM type 2 accounts 37.33% (n=112), followed by 35.67% of ACS (n=107), and 27% of AIS (n=81) (Table 2,3,4).

Comparison of platelet parameters between the DM type 2 group and the control group showed statistically significant increase in MPV and PC. When comparing the ACS group with the control group, our study showed statistically significant increase in MPV and PDW.

Comparison between the AIS group and control group showed a statistically significant increase in MPV and a decrease in PC.

Table 2. Comparison of various parameters between the patients with DM type 2 and the control group

Characteristic	DM type 2 patients	Control group	P-value
Number (n)	112	100	-
Age (years)	55.3 + 8.85	39.86 + 11.98	-
Male (%)	53 (47%)	51 (51%)	-
Female (%)	59 (53%)	49 (49%)	-
PC (x 109/L)	298.19 + 79.25	249.94 + 56.38	0.02
MPV (fL)	10.7 + 1.47	9.5 + 0.89	0.04
PDW (fL)	12.2 + 2.69	11.0 + 1.14	0.07
P-LCR (%)	20.9 + 8.4	20.5 + 2.99	0.09

Table 3. Comparison of various parameters between the ACS patients and the control group

Characteristic	ACS patients	Control group	P-value
Number (n)	107	100	-
Age (years)	64.76 + 8.89	39.86 + 11.98	-
Male (%)	50 (47%)	51 (51%)	-
Female (%)	57 (53%)	49 (49%)	-
PC (x 109/L)	387 + 122.3	249.94 + 56.38	0.09
MPV (fL)	10.15 + 1.05	9.5 + 0.89	0.02
PDW (fL)	12.35 + 0.93	11.0 + 1.14	0.03
P-LCR (%)	22.45 + 5.47	20.5 + 2.99	0.07

Table 4. Comparison of various parameters between the AIS patients and the control group

Characteristic	AIS patients	Control group	P-value
Number (n)	81	100	-
Age (years)	65.69 + 8.52	39.86 + 11.98	-
Male (%)	38 (47%)	51 (51%)	-
Female (%)	43 (53%)	49 (49%)	-
PC (x 109/L)	239.30 + 38.10	249.94 + 56.38	0.01

MPV (fL)	10.74 + 1.15	9.5 + 0.89	0.02
PDW (fL)	12.92 + 1.66	11.0 + 1.14	0.07
P-LCR (%)	25.26 + 6.46	20.5 + 2.99	0.09

Our study showed discrete arrangement of platelets in 90% of DM type 2, 88% of ACS, and 80% of AIS patients. Our study on PBS in various clinical conditions showed granularity of platelets in 84 % of DM type 2, 81% of ACS and 83% of AIS patients. In our study, the following observations were made regarding the percentage of large platelets on PBS in various clinical conditions, showing increased percentage of macrothrombocytes: 60% of DM type 2, 56% of ACS, and 52% of AIS patients (Table 5, Figure 1).

Table 5. Percentage of large platelets on PBS in non-hematological disorders

Non-hematological disorders	Absent	Normal (<5%)	Increased (>5%)
DM type 2 (n=112)	9 (8%)	36 (32%)	67 (60%)
ACS (n=107)	27 (25%)	20 (19%)	60 (56%)
AIS (n=81)	11 (14%)	28 (35%)	42 (52%)

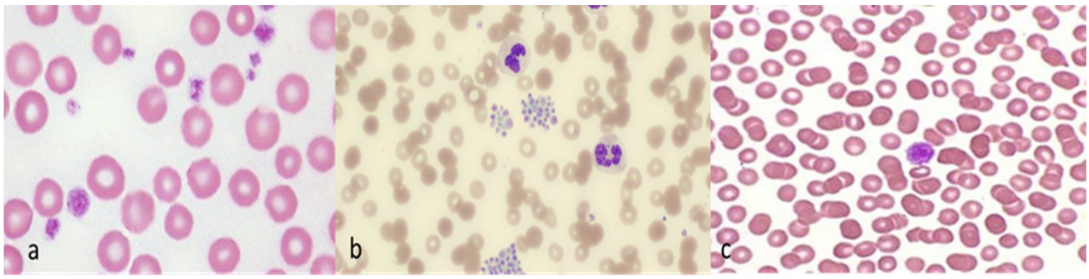

Fig. 1. a. Peripheral blood smear from a patient of DM type 2 showing large platelets. b. Peripheral blood smear from a patient of ACS showing platelet clumps. c. Peripheral blood smear from a patient of AIS showing thrombocytopenia and a macrothrombocyte

Discussion

The study consisted of 400 subjects documenting various platelet parameters. Platelet abnormalities were noted in patients with non-hematological disorders (DM type 2, ACS, and AIS).

High MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a significant role. Thus, DM has been considered as a “prothombotic state” with increased platelet reactivity. Platelet hyperactivity has been reported in diabetics and animals, both in vivo and in vitro (14).

Platelet activation contributes to the pathology by triggering thrombus formation and causing microcapillary embolization with the release of constrictive, oxidative, and mitogenic substances such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) that accelerate progression of local vascular lesions.

In our study, the platelet count in the diabetic group was higher than that of the control group, which was similar to the study done by Demirtuc et al. (15). Study by Hekimsoy et al. (16) had observed the opposite finding with lower platelet counts in the diabetic group compared with non-diabetic healthy subjects. Hence, the platelet count could be dependent on several variables, which is mean platelet survival, platelet production rate, and turnover rate in DM.

In our study, the diabetic group had significantly higher MPV than that of the control group. This agreed with the findings seen in studies done by Hekimsoy et al. (16) and Demirtune et al. (15). There was no statistically significant difference in PDW and P-LCR between Diabetic group and control group in our study.

In the majority of the DM type 2 cases, platelets on PBS were discrete (90%) and granular (84%). Hypogranularity was observed in few cases which could be due to larger platelets initiating aggregation or an artefactual change due to formation of clumps. The percentage of large platelets in these patients was increased beyond normal limits in 60% of cases, indicating that there is an increase in size of the platelets, supporting an increase in MPV.

Platelets have a significant role in the pathogenesis of ACS, where plaque rupture is followed by platelet activation and thrombus formation leading to coronary artery occlusion. The platelet–fibrinogen–platelet connection initiates the process of platelet aggregation and thus, leads to coronary thrombus formation and ACS. These findings led to the hypothesis that larger platelets as determined by their volumes (MPV) may be useful markers in patients with ACS.

It was found that patients with ACS tend to have significantly larger MPV similar to the studies done by Lippi et al. (17) and Khandekar et al. (18). The PDW was also significantly higher in patients with ACS than that in the control group in our study.

There was no statistically significant difference regarding the PC between the two groups that was similar to the observation found by Lippi et al. , while other studies done by Khandekar et al. (18) and Ridvan et al. (19) reported the PC to be lower while MPV to be higher in ACS patients compared to the controls. There was no statistically significant difference in P-LCR between patients with ACS and control group in our study.

In the majority of the ACS cases, platelets on PBS were discrete (88%) and granular (81%). Hypogranularity was observed in few cases, which could be due to larger platelets initiating aggregation or an artefactual change due to formation of clumps. The percentage of large platelets in these patients was increased beyond normal limits in 56% of cases, indicating that there is an increase in size of the platelets, supporting an increase in MPV.

This discrepancy in findings regarding the PC level and agreement on findings regarding the platelet volume parameters among different studies in ACS patients might indicate that the platelet volume indices rather than the platelet count are more important in determining the risk of developing ACS.

Platelets play an important role in the pathophysiology of ischemic stroke by developing intravascular thrombus after erosion or rupture of atherosclerotic plaques (20).

It had been shown that there is an association between MPV and ischemic stroke due to greater thrombopoietic reactivity of larger platelet (21).

PC is decreased in patients with AIS compared to control group, similar to the study done by Nadar et al. (22).

MPV is significantly increased in patients with AIS compared to control group similar to studies done by Greisenigger et al. (5), but in contrast to Tohgi et al. (12), which reported significantly diminish in MPV. There was no statistically significant difference in PDW and P-LCR between AIS and the control groups in our study.

In the majority of the AIS cases, platelets on PBS were discrete (80%) and granular (83%). Hypogranularity was observed in few cases, which could be due to larger platelets initiating aggregation or an artefactual change due to formation of clumps. The percentage of large platelets in these patients was increased beyond normal limits in 52% of cases compared to the control group, indicating that there was an increase in the size of the platelets, supporting an increase in MPV.

It has been found that patients that do badly (determined as death or dependency) have a significantly higher MPV in the acute phase of stroke than those who do well (independence) and tend to have a lower platelet count. It has also been found that MPV remained elevated 3 months post-stroke.

The significance of this has yet to be established, but since MPV measured 6 months post-acute myocardial infarction predicts recurrent coronary events and all-cause mortality, a persistently raised MPV following ischemic stroke may well be associated with recurrent vascular events and death.

With improvement in the technologies, advancement occurs in all fields including medicine. Automated cell counters are widely used for diagnosis of different diseases. At the present study, for analyzing platelet abnormalities, platelet parameters are utilized. Among them PC, MPV, PDW, and P-LCR are important parameters considered in our study.

In our study, the PC was higher and the MPV was lower in the anemic group compared to the control group. There was an inverse relationship noted between MPV and PC among anemic patients. The percentage of large platelets on PBS in these patients was not increased beyond normal limits, indicating that there is no increase in the size of platelets. A reduction of the total circulating red cell mass below normal limits may affect thrombopoiesis, leading to several changes in platelets, indicating the importance of evaluation of platelet parameters.

Our study showed that in DM type 2, PC was elevated along with MPV. The percentage of large platelets on PBS in these patients was increased beyond normal limits in 60% of cases, indicating that there is an increase in size of the platelets, supporting an increase in MPV.

The increased platelet size may be a useful factor in the increased risk of atherosclerosis associated with DM and associated vascular complications. Hence, MPV would be useful prognostic marker of cardiovascular complications in diabetes. Hence, we propose that MPV can be used as a simple and cost-effective tool to monitor the progression and control of DM and its cardiovascular complications.

Conclusion

MPV and PDW were significantly raised in the patients admitted with ACS. The percentage of large platelets on PBS in these patients was increased (>5%) in 56% of cases, indicating that there is an increase in the size of the platelets, supporting an increase in MPV.

In acute ischemic stroke, there was an elevation in MPV and a decrease in PC. The percentage of large platelets in these patients was increased beyond normal limits in 52% of cases when compared to the control group, indicating that there is an increase in the size of the platelets, supporting an increase in MPV.

In diabetes mellitus type 2, PC was elevated along with MPV. The percentage of large platelets on PBS was increased beyond normal limits in DM type 2, ACS, and AIS cases, indicating that there is an increase in the size of the platelets, supporting an increase in MPV.

The measurement of MPV levels may provide useful diagnostic and prognostic information to emergency physicians caring for patients with AIS. In the patients with suspected neurological ischemic symptoms, elevated levels may be considered as an atherosclerotic risk factor. These parameters are readily available, inexpensive, and useful markers which should be utilized with other investigational tools to screen patients presenting to the emergency room with chest pain who are suspected to have ACS.

Conflict of interest

The authors have no conflict of interest in this study.

Ethical Statement

The institutional ethical committee approved the study protocol. All the patients and healthy volunteers who participated in the study were informed about the purpose, procedure, benefits, and risks of the study and gave their written consent.

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