Platelet parameters: Can they serve as biomarkers of glycemic control or development of complications in evaluation of type 2 diabetes mellitus?

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Abstract:
BACKGROUND: Platelet function plays a crucial pathophysiological role in the development of atherothrombosis in patients with type 2 diabetes mellitus (DM). Platelet count (PC) and mean platelet volume (MPV) are simple, effective, and cheap tests that may be used to predict angiopathy in type 2 DM.

OBJECTIVES: The aims of this study were to analyze various platelet parameters including PC, plateletcrit (total mass of platelets) (PCT), and mean platelet indices that are MPV, platelet distribution width (PDW), and platelet-large cell ratio (PLCR) in the type 2 DM patients, to compare various platelet indices between DM patients (with and without complications) and controls.

MATERIALS AND METHODS: This was a cross-sectional study conducted over a period of 3 months. Complete blood count along with blood glucose and HbA1c was estimated. The study population was divided into three groups: Group 1: Normal controls (n = 30); Group 2: DM patients without complications (n = 30); and Group 3: DM patients with complications (n = 30). Based on HbA1c levels among the diabetic patients, the diabetic groups were also classified as DM with HbA1c <7% and DM with HbA1c >7%.

RESULTS: All the platelet parameters were found to be higher among DM with complication as compared to DM without complication, and this was found to be statistically significant. Among the platelet parameters, MPV, PCT, and PDW were found to be higher among DM with HbA1c >7% as compared to DM with HbA1c <7%, and this was found to be statistically significant while there was no significant differences in PC and PLCR between the two groups.

CONCLUSION: Monitoring of DM to prevent the occurrence of vascular complications is the need of the hour. The results of the study suggest a role of various platelet indices as a simple and cost-effective tool to monitor the progression and control of DM.

Keywords:
Diabetes mellitus, glycosylated hemoglobin, platelet parameters

Introduction

Diabetes mellitus (DM) is a metabolic disorder which is a major global health problem on account of its high prevalence as well as morbidity.[1] According to the International Diabetes Federation, as of 2014, worldwide, 387 million people were suffering from diabetes. India has the highest burden of diabetic patients.[2]

Chronic hyperglycemia results in micro- and macrovascular complications in patients with type 2 DM. The increased platelet activity has been implicated as a factor in the development of vascular complications.
in this metabolic disorder. Moreover, the function of platelets seems to be related to their sizes as large platelets are more reactive and contain high amount of dense granules and present increased thrombotic potential as shown by some authors.

A gamut of potential risk factors for type 2 diabetes have emerged from various studies in the literature including lifestyle risk factors, inflammatory markers, metabolic derangements, and genetic risk factors. Out of these, many have been found to be independently associated with type 2 diabetes. Platelet function plays a crucial pathophysiological role in the development of atherothrombosis in patients with type 2 DM. This has been reported by many authors that increased morbidity and mortality in type 2 DM are associated with macrovascular (cardiovascular diseases, stroke, and peripheral arterial disease) and microvascular (nephropathy, neuropathy, and retinopathy) complications due to platelet dysfunction. Platelet count (PC) and mean platelet volume (MPV) are simple, effective, and cheap tests that may be used to predict angiopathy in type 2 DM. Elevated MPV has been documented to predict bad outcome for acute ischemic cerebrovascular events independent of other clinical parameters.

The different parameters which represent the condition of platelets are PC, plateletcrit (total mass of platelets) (PCT), and mean platelet indices that are MPV, platelet distribution width (PDW), and platelet-large cell ratio (PLCR). Among these, MPV is most extensively researched and is a reflection of the average size of platelets. MPV has been found to increase in myocardial infarction, coronary artery disease, as well as DM. Platelet indices which reflect platelet morphology, namely, PDW, PLCR, and PCT also play a significant role in atherosclerosis and thrombosis.

The present study was conducted to analyze the role of various platelet parameters (PC, PCT, MPV, PLCR, and PDW) in type 2 DM patients and to assess the correlation between fasting blood glucose, glycaemic control, microvascular complications, and platelet indices.

Materials and Methods

The present study was conducted in the Department of Pathology, ESIC Medical College, Faridabad. Ethical clearance was obtained from the Institutional Ethics Committee, and written informed consent was taken from all the patients.

This was a cross-sectional study comprising 60 DM (type 2) patients attending medicine clinics (outpatient department) and 30 nondiabetic controls. Out of the 60 DM patients, 30 were DM without complications while 30 were DM with any microvascular complications of diabetes including nephropathy, neuropathy, microangiopathy, or retinopathy. The study was conducted over a 3-month period from July 2017 to September 2017. All the patients who met the inclusion criteria and those who gave consent were included in the study. The demographic information and clinical details of the patients were recorded including fasting blood sugar, duration of diabetes, family history of diabetics, hypertension, drug history, special reference to any complications, or comorbidities.

Inclusion criteria

All noninsulin-dependent DM (type 2 DM) patients on treatment attending the medicine clinics were included in the study.

Exclusion criteria

1. Nutritional anemia can be a cause of reactive thrombocytosis, thereby increased MPV, so male patients with hemoglobin (Hb) <13 g% and female patients with Hb <12 g% were excluded from the study.
2. Control group – Nondiabetics with coronary artery disease were not taken as controls.
3. Diabetics on anti-platelet drugs such as aspirin and clopidogrel or on insulin were excluded.
4. Patients with any diagnosed malignancy/thrombocytopenia/thrombocytosis were excluded from the study.

Sample collection

Venous blood samples were collected in the potassium ethylenediaminetetraacetic acid and fluoride vacutainers for estimation of hematological indices and blood glucose, respectively. Samples were tested within 1 h of collection to minimize variations. Complete blood count was performed on 5-part hematology analyzer (Sysmex XN 1000). Blood glucose and HbA1c were estimated using fully automated biochemistry analyzer (Randox Daytona).

The study population was divided into three groups:

Group 1: Normal controls (nondiabetics) (n = 30);
Group 2: DM patients without complications (n = 30); and Group 3: DM patients with complications (n = 30). Based on HbA1c levels among the diabetic patients, the diabetic groups were also classified as DM with HbA1c <7% and DM with HbA1c >7%.

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17 software for Windows (SPSS Inc., Chicago, IL, USA). The results are presented in mean ± standard deviation. Statistical tests such as t-test, analysis of variance (ANOVA),
and Mann–Whitney U-test were applied to evaluate the statistical significance and correlation of different parameters in the various groups (normal control, DM without complications, DM with complications, and DM with HbA1c <7% and DM with HbA1c >7%). \( P \leq 0.05 \) was considered significant.

**Observations and Results**

The study comprised three groups: Group 1: Normal controls (nondiabetics) \((n = 30)\); Group 2: DM patients without complications \((n = 30)\); and Group 3: DM patients with complications \((n = 30)\). In Group 3 (DM with complications), 23 patients had retinopathy, 13 had neuropathy, 10 had microangiopathy, and 8 had nephropathy with many patients suffering from more than one complication. Moreover, on reclassifying the DM groups based on HbA1c levels, there were two groups: DM with HbA1c <7% \((n = 20)\) and DM with HbA1c >7% \((n = 40)\). The distribution of the study groups is shown in Figures 1 and 2.

Patients having diabetes with complication had a higher mean age as compared to patients having diabetes without complication \((55.63 \pm 7.49 \text{ vs. } 50.87 \pm 9.75)\), and this was found to be statistically significant \((P = 0.038)\). The mean duration of diabetes (in years) in patients without complications was lower compared to those with complications \((2.44 \pm 2.03 \text{ vs. } 8.12 \pm 5.09)\), the difference being statistically significant. Creatinine and HbA1c were found to be higher among patients with complication as compared to patients without complication, and this was found to be statistically significant. A comparison of clinical and biochemical parameters between DM without complications and DM with complications is shown in Table 1 and Figure 3.

Hb was found to be higher among patients without complication as compared to patients with complications \((P = 0.002)\). All the platelet parameters including PC, MPV, PDW, PLCR, and PCT were found to be higher among DM with complication as compared to DM without complication, and this was found to be statistically significant. Table 2 and Figure 4 depict comparison of hematological parameters between DM without complications and DM with complications.

For comparison between the three groups, one-way ANOVA test was applied. A statistically significant difference in Hb as well as all the platelet parameters was found between the three groups as shown in Table 3 and Figure 5.

To compare the two groups, DM with HbA1c ≤7% \((n = 20)\) and DM with HbA1c >7% \((n = 40)\), Mann–Whitney U-test was applied. Fasting and postprandial blood sugar and triglyceride levels were found to be higher among DM with HbA1c >7% as compared to DM with HbA1c <7%, and this was found to be statistically significant [Table 4].
Among the platelet parameters, MPV, PCT, and PDW were found to be higher among DM with HbA1c >7% as compared to DM with HbA1c <7%, and this was found to be statistically significant while there was no significant differences in PC and PLCR between the two groups. Table 5 and Figure 6 display the comparison of hematological parameters between DM with HbA1c <7% and DM with HbA1c >7%.

**Discussion**

DM is characterized by a prothrombotic state comprising increased platelet activation and coagulation proteins and reduced fibrinolysis. This is followed by the development of cardiovascular and atherosclerotic complications associated with DM. The prevalence of type 2 diabetes is on a rise globally and poses a

| Variable      | Mean±SD | P     |
|---------------|---------|-------|
| Age (years)   | 50.87±9.75 | 55.63±7.49 | 0.038 |
| Weight (kg)   | 54.77±9.54 | 54.00±8.75 | 0.747 |
| Height (feet) | 5.180±0.28 | 5.163±0.33 | 0.834 |
| BMI (kg/m²)   | 22.71±3.70 | 21.84±3.73 | 0.368 |
| Duration of diabetes (years) | 2.441±2.02 | 8.116±5.09 | 0.00001 |
| HbA1c (%)     | 7.847±1.56 | 9.790±2.24 | 0.000 |
| Fasting blood sugar (mg/dl) | 161.57±67.97 | 173.00±41.62 | 0.436 |
| Postprandial blood sugar (mg/dl) | 237.80±89.61 | 253.23±70.104 | 0.461 |
| Total cholesterol (mg/dl) | 180.63±53.73 | 186.43±52.053 | 0.673 |
| HDL (mg/dl)   | 44.00±15.47 | 41.80±7.355 | 0.486 |
| Triglycerides (mg/dl) | 240.67±170.71 | 200.83±73.801 | 0.248 |
| Urea (mg/dl)  | 27.30±9.95 | 30.87±11.069 | 0.194 |
| Creatinine (mg/dl) | 0.820±0.18 | 1.087±0.42 | 0.003 |

T-test: P<0.05 was considered significant at 95% CI. BMI=Body mass index, HbA1c=Glycated hemoglobin, HDL=High-density lipoprotein, DM=Diabetes mellitus, SD=Standard deviation, CI=Confidence interval

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**Figure 4:** Bar chart depicting comparison of platelet parameters between diabetes mellitus without complications and diabetes mellitus with complications

**Figure 5:** Bar chart depicting comparison of platelet parameters between the three groups: normal control, diabetes mellitus without complications, and diabetes mellitus with complications

**Figure 6:** Bar chart depicting comparison of platelet parameters between the two groups: diabetes mellitus with HbA1c <7% and diabetes mellitus with HbA1c >7%.
Table 3: Comparison of hematological parameters among the three groups, that is, normal controls, diabetes mellitus without complications, and diabetes mellitus with complications

| Variable       | Control (n=30) Mean±SD | DM without complications (n=30) Mean±SD | DM with complications (n=30) Mean±SD | F statistic | P    |
|----------------|------------------------|----------------------------------------|------------------------------------|-------------|------|
| Hb (g/dl)      | 13.080±1.3105          | 13.633±0.8774                         | 12.883±0.9289                      | 4.063       | 0.021|
| TLC (cummm)    | 9527.33±2665.854       | 8787.67±3514.914                      | 8563.33±2476.579                   | 0.895       | 0.412|
| Plt count (×10^5/cummm) | 2.6503±0.78556 | 2.6653±0.50630                        | 3.0463±0.61508                      | 3.621       | 0.031|
| MPV (fl)       | 12.0467±1.53212        | 11.8333±1.16599                       | 9.4667±1.24355                     | 35.073      | <0.05|
| PLCR (%)       | 41.147±11.9963         | 34.450±6.6954                         | 25.230±4.4630                      | 27.547      | 0.000|
| Plateletcrit (%) | 0.3123±0.07934    | 0.3133±0.0678                         | 0.1900±0.06237                     | 30.614      | 0.000|
| PDW (fl)       | 16.057±3.9991          | 16.543±1.6831                         | 19.827±1.9771                      | 16.646      | 0.000|

One-way ANOVA test: P<0.05 was considered significant at 95% CI. Hb=Hemoglobin, TLC=Total leukocyte count, Plt=Platelets, MPV=Mean platelet volume, PLCR=Platelet-large cell ratio, PDW=Platelet distribution width, DM=Diabetes mellitus, fl=Femtoliter, ANOVA=Analysis of variance, SD=Standard deviation, CI=Confidence interval.

Table 4: Comparison of clinical and biochemical parameters between diabetes mellitus with glycated hemoglobin ≤7% and diabetes mellitus with glycated hemoglobin >7%

| Variable                 | HbA1c ≤7% (n=20) Mean rank | HbA1c >7% (n=40) Mean rank | Mann-Whitney U-test | P    |
|-------------------------|-----------------------------|-----------------------------|---------------------|------|
| Age (years)             | 31.11                       | 29.28                       | 375.5               | 0.70 |
| Weight (kg)             | 30.79                       | 29.93                       | 388.5               | 0.856|
| Height (feet)           | 30.73                       | 30.05                       | 391                 | 0.883|
| Fasting blood sugar (mg/dl) | 37.70                     | 16.10                       | 112                 | <0.05|
| Postprandial blood sugar (mg/dl) | 35.11                     | 21.28                       | 215                 | 0.004|
| Total cholesterol (mg/dl) | 32.76                      | 25.98                       | 309.5               | 0.156|
| HDL (mg/dl)             | 31.70                       | 28.10                       | 352                 | 0.451|
| Urea (mg/dl)            | 31.40                       | 28.70                       | 364                 | 0.572|
| Creatinine (mg/dl)      | 30.60                       | 30.30                       | 396                 | 0.949|

Mann–Whitney U-test: P<0.05 was considered significant at 95% CI. HbA1c=Glycated hemoglobin, HDL=High-density lipoprotein, CI=Confidence interval.

Table 5: Comparison of hematological parameters between diabetes mellitus with glycated hemoglobin ≤7% and diabetes mellitus with glycated hemoglobin >7%

| Variable       | HbA1c >7% (n=40) Mean rank | HbA1c ≤7% (n=20) Mean rank | Mann-Whitney U-test | P    |
|----------------|-----------------------------|-----------------------------|---------------------|------|
| Hb (g/dl)      | 30.43                       | 30.65                       | 397                 | 0.962|
| TLC (cummm)    | 31.43                       | 28.65                       | 363                 | 0.561|
| Plt count (×10^5/cummm) | 32.66                     | 26.18                       | 313.5               | 0.175|
| MPV (fl)       | 36.71                       | 18.08                       | 151.5               | 0.000|
| PLCR (%)       | 32.46                       | 26.58                       | 321.5               | 0.218|
| Plateletcrit (%) | 34.21                      | 23.08                       | 251.5               | 0.020|
| PDW (fl)       | 35.23                       | 21.05                       | 211                 | 0.003|

Mann–Whitney U-test: P<0.05 was considered significant at 95% CI. Hb=Hemoglobin, TLC=Total leukocyte count, Plt=Platelets, MPV=Mean platelet volume, PLCR=Platelet-large cell ratio, PDW=Platelet distribution width, DM=Diabetes mellitus, fl=Femtoliter, HbA1c=Glycated hemoglobin, CI=Confidence interval.

challenge on the health-care system as well as on the public health and socioeconomic development of the countries. The prevalence of diabetes was estimated to be 387 million worldwide as of 2014. Moreover, in 2014 alone, 4.9 million deaths have been caused due to diabetes and its complications.[18]

The prevalence of microvascular complications of diabetes is higher in diabetics with poor glycemc control, longer duration of the disease, associated hypertension, and obesity.[19] This results in a deadly combination of morbidities and mortalities in DM. A gamut of potential risk factors for type 2 diabetes have emerged from various studies[6] in the literature including lifestyle risk factors, inflammatory markers, metabolic derangements, and genetic risk factors which may serve as markers for identification of high-risk groups so that the preventive approaches may be focused on such groups to derive maximal benefit.

Platelet function plays a significant role in the development of atherothrombosis in patients with type 2 DM. This has been documented by several authors that platelet dysfunction is responsible for increased morbidity and mortality in type 2 DM associated with macrovascular (cardiovascular diseases, stroke, and peripheral arterial disease) and microvascular (nephropathy, neuropathy, and retinopathy) complications.[7,8] Moreover, platelet size seems to be related to their function as MPV has been found to be higher in diabetics, especially complicated cases.[20-25]
The present study was conducted to study the role of platelet parameters in DM in terms of glycemic control and development of complications.

All the platelet parameters including PC, MPV, PDW, PLCR, and PCT were found to be higher among DM with complication as compared to DM without complication, and this was found to be statistically significant. These findings are in accordance with most of the studies in the literature like Demirtas et al., Ashraf et al. while several others found significant differences in some parameters not in others, namely, Yilmaz and Yilmaz. Mousa et al., Erdogan et al. found a positive correlation between MPV, PDW with DM not with PC, PCT, and PLCR; Raman and Kundur observed a significant association of PC and PDW with DM while Buch et al. found a positive association of MPV, PDW with DM but not with PLCR, and PC.

On the contrary, a few authors did not find any correlation between platelet parameters and DM while Akinsegun et al. observed a statistically significant difference in PCs of diabetics and healthy controls while none existed between MPV in diabetics and healthy controls.

A statistically significant difference in Hb as well as all the platelet parameters was found between the three groups, that is, normal controls, DM without complications, and DM with complications similar to several other researchers.

Among the platelet parameters, MPV, PCT, and PDW were found to be higher among DM with HbA1c >7% as compared to DM with HbA1c <7%, and this was found to be statistically significant while there was no significant differences in PC and PLCR between the two groups. These results are quite similar to Shukla et al. and Demirtas et al. observed an increase in PCT, MPV, and PDW in the DM and control groups as well as higher values among patients with complications of DM. MPV has been documented to show a positive correlation with higher HbA1c values by many authors. This is in stark contrast to the observations of Hasan et al. and Sulochana et al. who did not observe any significant relation of platelet indices in diabetic patients with high glycated hemoglobin.

Diabetes and its vascular complications can become a financial burden and affect a country’s economic growth, especially in developing countries like India with the highest number of diabetics. Therefore, the need of the hour is monitoring of DM to prevent the occurrence of vascular complications as these are constantly increasing day by day. Platelet indices may serve as useful, simple, and cost-effective markers for development of complications in diabetic patients and thereby may play a crucial role in monitoring of DM.

**Conclusion**

All the platelet parameters including PC, MPV, PDW, PLCR, and PCT were found to be higher among DM with complication as compared to DM without complication, and this was found to be statistically significant. Among the platelet parameters, MPV, PCT, and PDW were found to be higher among DM with HbA1c >7% as compared to DM with HbA1c <7%, and this was found to be statistically significant. Between the two groups.

Future multi-institutional studies involving larger number of patients will be required to precisely define the status of platelet parameters in DM. The results, however, are encouraging and suggest a role of various platelet indices as a simple and cost-effective tool to monitor the progression and control of DM.

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**Conflicts of interest**

There are no conflicts of interest

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