Original Article

A Cross-Sectional Analytical Study of Platelet Indices in Type 2 Diabetes Mellitus

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Abstract

Background: Diabetes mellitus (DM) is a growing public health burden in India, projected to become the 'diabetic capital' of the world by 2030. Platelet activation plays a key role in the pro-thrombotic state associated with DM. Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and Platelet Large Cell Ratio (P-LCR) are potential biomarkers to evaluate platelet activity and predict diabetic complications.
Methods: A cross-sectional, single-visit analytical study were conducted at a tertiary care center in Bhuj, Gujarat, India between 1st June 2023 to 30th November 2024.
Results: A total number of 130 cases each of non-diabetic and diabetic cases with or without complications were studied. The mean of MPV, PDW and P-LCR were statistically significantly increased in diabetic patients with complications than diabetic patients without complications and non-diabetic volunteer group. Poor glycemic control and raised FBS and PPBS blood sugar level cause increased risk of diabetic complications. However, no statistically significant differences were observed in RBS among all the study groups. Diabetic patients were divided into two groups according to their HbA1c levels: 3.1% in group A1 (mean HbA1c $<$6.5%) and 96.9% in group A2 (mean HbA1c $\geq$6.5%). However, platelet indices and blood sugar parameters in diabetes were not statistically significant between groups A1&A2.
Conclusion: Our study suggests that increased platelet volume indices may contribute to the prothrombotic state in diabetes mellitus. The raised MPV, PDW and P-LCR can be considered as key biomarkers for early detection of impending diabetic complications.

Keywords: Platelet indices; Diabetes-mellitus; HbA1C level

Introduction

Platelets are important hematological cells derived from the megakaryocytes in bone marrow. Platelets play important roles in several diverse processes beyond hemostasis and thrombosis, including promoting inflammatory and immune responses, maintaining vascular integrity, and contributing to wound healing. Platelets produce, store and release pro-inflammatory, anti-inflammatory and antigenic factors. These factors contribute to atherosclerosis, sepsis, acute lung injury, vascular re-stenosis, hepatitis [1, 2]. Diabetes mellitus (DM) is a major global health problem [3]. WHO projects that diabetes will be 7th leading cause of death in 2030 [4]. Type 2 DM account for 80% of all DM cases [5]. According to WHO, India will be the country with the maximum number of diabetic patients in the world by 2030. In 2014, 40.9 million people were affected with diabetes in India and the projected estimate for the year 2030 is 80 million [6].

Diabetes mellitus is a pro-thrombotic state which is characterized by chronic hyperglycaemia, dyslipidaemia and insulin resistance. The enhanced platelet morphology and function in diabetic condition contribute to prothrombotic diseased state [7]. Macro and microvascular complications are a major cause of morbidity and mortality in type 2 diabetic patients [8].

It has been reported that platelets from diabetic patients synthesize more prothrombotic factors such as thromboxane A2 than normal platelets [9].

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 [10].

An increased platelet count and activity have been reported in diabetes as demonstrated by increase in GP IIb/IIIa, Ib/IX, Ia/IIa, CD 62 and CD 63 [11, 12] and is not influenced by glycemic control [10]. An activated megakaryocyte-platelet system in diabetes has been reported to be responsible for larger than normal platelets circulating in DM patients [10].

Mean platelet volume (MPV) is an indicator of average size and activity of the platelets and is reported to be higher in diabetes mellitus and is considered as a risk factor for heart disease. Similarly, 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 [13].

The normal range of mean platelet volume is 8.6 - 15.5 fl and it is influenced by the total platelet count [14].

Platelet distribution width (PDW) is a commonly used parameter that signifies the changes in the platelet size which has a wide range. The normal range of PDW is from 8.3 to 25 fl [15, 16].

Materials and Methods

A cross-sectional analytical study was undertaken at tertiary care hospital at Bhuj, Gujarat, India. 130 participants were included each in Group A and Group B respectively. Group B were healthy non-diabetic volunteers while Group A were diabetic patients admitted in hospital. All participants were enrolled after filing of consent forms for study and case proforma were then filled by the principal author.

The prevalence rate of diabetes mellitus in India were taken as 9.3%. Using a 95% confidence interval and 5% allowable error, sample size were calculated as follows: n = [1 $\times$ 1000000 $\times$ 0.093 $\times$ (1 - 0.093)] / [(5^2 / 1.96^2 $\times$ (1000000 - 1) + 0.093 $\times$ (1 - 0.093))] $\approx$ 130 participants per group. Final sample size: 130 diabetic patients and 130 non-diabetic individuals.

(Wherein: Design effect (for cluster surveys- DEFF) :1 Population size (N)= 1000000 Hypothesized % frequency of outcome factor in the population (p): 9.3% [21] $\pm$5 Q= 1-p Confidence limit as % of 100 (absolute $\pm$ %) (d): 5% Person- time variable (z): 1.96 α= Inter observer variable)

Simple random sampling method were the participants' recruitment procedure. The duration of study was from 1st June 2023 to 30th November 2024 (18 months).

Inclusion criteria:

Type 2 Diabetics admitted as indoor patients. All type-2 diabetic patients were grouped as Group A and were compared with equal number of randomly selected, sex-matched, non-diabetic individuals' controls (Group B).

Exclusion criteria:

Subjects on antiplatelet drugs such as aspirin and clopidogrel. Subjects with any diagnosed malignancy. Patients who were not willing to give consent. Patients with haemoglobin less than 9 gm%.

Method:

The blood samples of the patients were drawn from the antecubital vein using a 5-ml syringe and immediately mixed in EDTA vacutainers. The EDTA sample were run within 2 hours of venipuncture using the seven-part differentiated automated Hematology analyzer Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan) and complete blood count analysis of the sample were made including the platelet indices (MPV, PDW and P-LCR). Peripheral smears (Field-stained smears) were evaluated for platelet count and for platelet morphology.

Note on Preanalytical Variables:

Mean Platelet Volume (MPV) is known to increase with EDTA storage time due to platelet swelling. To minimize variability, all samples in this study were analyzed within 2 hours of collection. The Sysmex XN-1000 Hematology Analyzer used

in this study were calibrated regularly and internal quality control (QC) checks were performed daily to ensure analytical accuracy. MPV is known to increase over time due to platelet swelling in EDTA-anticoagulated samples. To minimize this preanalytical variability, all EDTA samples in the current study were processed within 2 hours of collection. Moreover, the Sysmex XN-1000 hematology analyzer used for platelet indices measurement were subjected to daily internal quality control (QC) checks using manufacturer-provided control materials, and routine calibration procedures were carried out in accordance with ISO/NABL standards to ensure accuracy and reproducibility.

Cut off values of platelet indices were derived by calculating the minimum value of these indices in the diabetic cases. All the diabetic and non-diabetic subjects were interviewed as per the prepared proforma which included the complete clinical evaluation with specific reference to any associated macrovascular or microvascular complications as well as any drugs taken.

Relevant investigations like blood glucose levels and HbA1c level were performed for confirmation of the diagnosis. The instruments to be used for HbA1c and other glucose parameters were Bio-rad and Vitros respectively.

After baseline evaluation, diabetic patients (Group A) were divided into two groups according to their HbA1c levels: group A1 consisted of patients with HbA1c levels <6.5% and group A2 which consisted of patients with HbA1c levels ≥6.5%. (The latest HbA1c cut-off for diabetic range were considered according to American Diabetic Association 2016 criteria.)

Data were finally entered in Microsoft excel sheet and analyzed using appropriate statistical tests by suitable SPSS software.

Results

The statistical results are represented in Table 1 and subsequent comparisons in Tables 2 through 4. The graphical distribution of platelet indices and blood sugar levels is presented in Figure 1 and Figure 2 respectively.

In present study, total of 130 diabetic patients (86 males, 44 females) and 130 controls (74 males, 56 females) were selected. The maximum number of diabetic males were seen in age group of 41–60 years.

Comparison of platelet indices were made in cases and controls.

The blood glucose parameters (FBS, RBS, PPBS and HbA1c) were statistically significantly higher in diabetic patients compared to the non-diabetic individuals. The MPV, PDW and P-LCR were evaluated in diabetic and non-diabetic population.

The mean MPV in diabetic cases were 10.6 ± 1.3 fl compared to 9.99 ± 1.05 fl in non-diabetic individuals with p value 0.0001. Mean PDW and P-LCR in diabetic patients were 12.3 ± 3.2 fl and 28.96 ± 9.8% compared to non-diabetic individuals where it were 11.1 ± 2.5 fl and 24.1 ± 6.98% respectively. Our study observed p value of MPV, PDW and P-LCR to be highly significant in diabetic patients ($p < 0.05$) (Table 1).

Platelet indices were also compared in diabetic patients with complications, without complications and non-diabetic individuals. The mean of MPV, PDW and P-LCR were statistically significantly increased in diabetic patients with complications (10.9 ± 1.1, 12.6 ± 2.6 fl and 30.5 ± 7.2%) than diabetic patients without complications (10.5 ± 1.4, 12.2 ± 3.5 fl and 28.4 ± 10.4%) and nondiabetic group (9.99 ± 1.1, 11.1 ± 2.5 fl and 24.1 ± 6.98%) (Figure 1).

Our study reinforced the fact that poor glycemic control and raised FBS and PPBS blood sugar level cause increased risk of diabetic complications. However, no statistically significant difference were observed in RBS among all the three study groups [As p value of RBS= 0.283 (>0.05)] (Figure 2).

Comparison of diabetic cases in group A1 and group A2: After baseline evaluation, diabetic patients were divided into two groups according to their HbA1c levels: group A1 consisted of patients with HbA1c levels <6.5% and group A2 consisted of patients with HbA1c levels ≥6.5%. The latest HbA1c cut-off for diabetic range were considered according to American Diabetic Association 2016 criteria. Out of 130 Type 2 DM cases, there were 04 patients (3.1%) in group A1 (mean HbA1c <6.5%) and 126 patients (96.9%) in group A2 (mean HbA1c ≥ 6.5%). Our study observed that platelet indices and blood sugar parameters in diabetes were not statistically significant between groups A1&A2 (p value >0.05), due to fewer A2 subjects in study duration. The equal number of A1 & A2 grouped subjects which are gender-matched can help evaluate the better results of statistical significance and state its importance.

Discussion

The observed increase in platelet indices in diabetic patients may be mechanistically linked to the underlying pathophysiology of insulin resistance. Insulin has inhibitory effects on platelet activation under normal physiological conditions. In type 2 diabetes, insulin resistance leads to hyperinsulinemia, which in turn promotes megakaryocyte hyperactivity and the production of larger, more reactive platelets. This contributes to the elevated MPV, PDW, and P-LCR observed in our study. Furthermore, hyperglycemia induces oxidative stress and glycation of platelet surface proteins, further amplifying platelet

Table 1: Comparison of means of blood sugar and platelet indices among diabetic cases and non-diabetic controls

Parameters	**Diabetic (n=130)**	**Mean ± 2SD**	**Non-diabetic (n=130)**	**Mean ± 2SD**	**'p' value (2 tailed)**
HbA1c (%)	9.2	±1.9	5.4	±0.5	<0.005
FBS (mg/dl)	173.8	±64.9	91.9	±14.1	0.0003
RBS (mg/dl)	236.3	±114.2	100.6	±20.9	<0.005
PPBS (mg/dl)	280.3	±88.03	119.8	±10.5	0.005
MPV (fl)	10.6	±1.3	9.99	±1.05	0.0001
PDW (fl)	12.3	±3.2	11.1	±2.5	0.0006
P-LCR (%)	28.96	±9.8	24.1	±6.98	<0.005

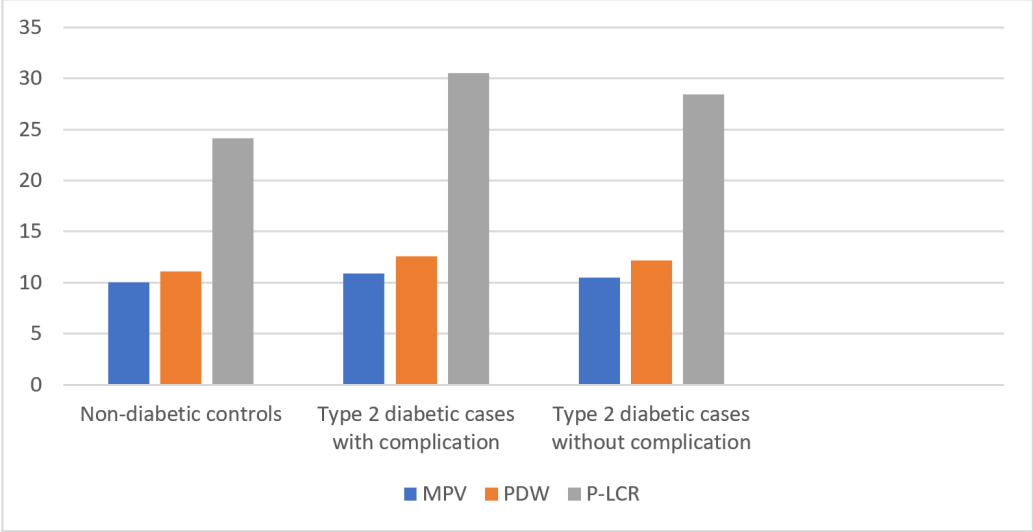

Figure 1: Graph showing Comparison of platelet indices in diabetic patients with complications, without complications and non-diabetic individuals

reactivity and aggregation potential. This mechanistic pathway aligns with the prothrombotic state seen in poorly controlled diabetic patients [1, 2, 3, 4].

India leads the world with the largest number of diabetic patients. Also, the morbidity and complications associated with diabetes are well known. This warrants a detailed knowledge of pathogenesis of the disease. The aim of this study was to evaluate the platelet activity in diabetic patients. Larger platelets are younger, more reactive and have greater potential to aggregate. High MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role. One possible mechanism of increased MPV in DM is osmotic swelling due to raised blood glucose and perhaps due to a shorter life span of platelets in diabetic patients [17].

According to our study, the blood glucose parameters (FBS, RBS, PPBS and HbA1c) were statistically significantly higher in diabetic patients compared to the non-diabetic individuals (p value<0.001).

The MPV, PDW and P-LCR were evaluated in diabetic and non-diabetic population. The p value of MPV, PDW and P-LCR were highly significant in diabetic patients ($p<0.05$) and mean of MPV, PDW and P-LCR were statistically significantly increased in diabetic patients with complications than diabetic patients without complications and non-diabetic group.

Our study reinforced the fact that poor glycemic control and raised FBS with high PPBS blood sugar level cause increased risk of diabetic complications. However, no statistically significant differences were observed in RBS among all the three study groups [As p value of RBS= 0.283 (>0.05)].

Comparison of MPV with other studies: The main goal of this study were to determine platelet indices in non-diabetic

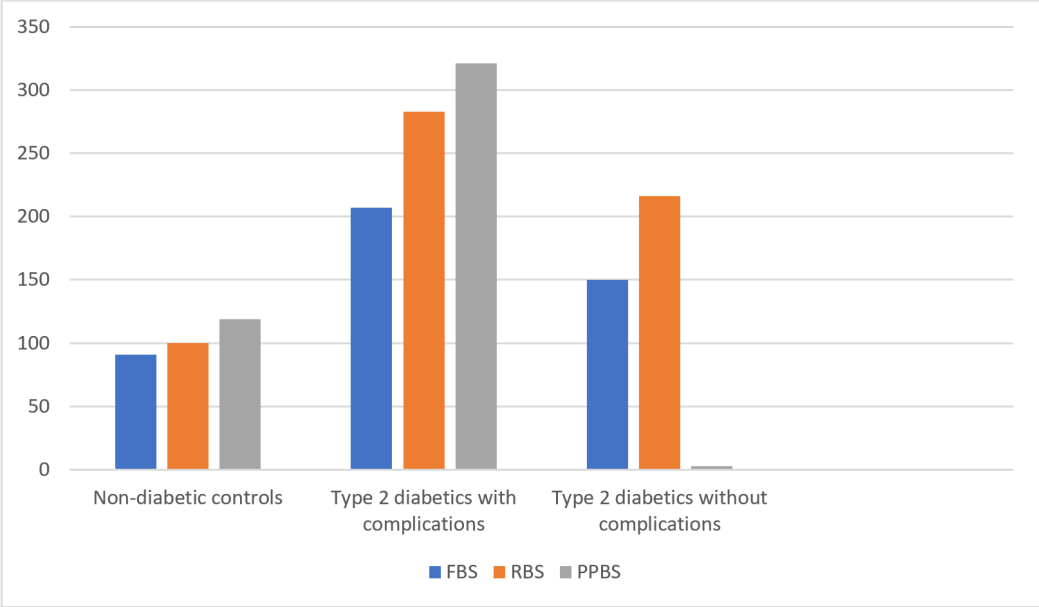

Figure 2: Graph showing Comparison of blood sugar level in diabetic patients with complications, without complications and non-diabetic individuals

individuals and diabetic patients. In our study, the mean of MPV, PDW and P-LCR were statistically significantly increased in diabetic patients with complications (10.9 ± 1.1, 12.6 ± 2.6 fl and 30.5 ± 7.2%) than diabetic patients without complications (10.5 ± 1.4, 12.2 ± 3.5 fl and 28.4 ± 10.4%) than non-diabetic group (9.99 ± 1.1, 11.1 ± 2.5 fl and 24.1 ± 6.98%). MPV in type 2 diabetic patients with complications were 10.9±1.1. This difference were statistically significant (p value<0.0001), thus reinforcing the concept that platelet activity, reflected by mean platelet volume is higher in diabetic patients, compared to non-diabetic individuals. These findings were in agreement with several studies done over the last few years.

Walinjkar, et al. [18] showed that MPV were significantly higher in diabetic patients (11.32±1.72 fL), compared to non-diabetic individuals (8.56±1.51 fL) with a p-value of 0.0001. Moreover, the increase in MPV were more significant in diabetic subjects with microvascular complications when compared with those without microvascular complications (Table 2).

Shilpi, et al. [19] showed that MPV were significantly higher in diabetic patients compared to non-diabetic individuals (11.3 ± 1.0 vs. 9.0 ± 0.6). The average age of presentation with type 2 diabetes mellitus were 53 ± 5.7 years. The mean duration of diabetes were 4.7 ± 2.5 years (Table 2).

Kodiatte, et al. [17] demonstrated that MPV were higher in diabetic patients (8.29±0.735 fL), when compared to non-diabetic individuals (7.47±0.73 fL) [p value < 0.001] (Table 2).

Vadatti, et al. [20] demonstrated that MPV were significantly higher in diabetic patients, when compared to non-diabetic individuals (7.91 ± 0.87 fL vs 6.91 ± 0.71 fL respectively [p value < 0.00001] (Table 2).

Moreover, another platelet parameter PDW were also significantly higher in diabetic patients compared to controls. Similar results were noted in other studies done by Alhadas, et al. [21]; Jabeen, et al. [22] and Demirtas, et al. [23] with significantly higher PDW levels among diabetic cases. Among the diabetic patients, only PDW were significantly higher in those with complications as compared to those without (P = 0.006) (as per Table 3).

Alhadas, et al. [21] studied 352 type 2 DM patients. 117 (33.2%) of participants were suffering from at least one microvascular complication. The platelet distribution width (PDW) were significantly increased in DM patients with complications as compared to without complications (16.57±2.49fl vs. 14.97±2.41fl) (P<0.001).

Demirtas, et al. [23] observed 307 diabetic patients (124 male, 183 females; mean age 50.8±8.5), and 187 controls (76 male, 111 female; mean age 51.1±10.1). In their study, they showed an increase in platelet distribution width (PDW): 17.8 ± 1.06 fl vs 17.5 ± 0.87 fl (p = 0.039) in the DM and control groups, respectively.

Jabeen, et al. [22] evaluated 170 Diabetic patients (Type-2) (93 male & 77 females) and 92 healthy control (42 male & 50 females). Mean age of controls were 45.5±0.77 years and 51.08±0.7 years for diabetic patients. Significant increase in PDW (p < 0.0033), were found in diabetic patients as compared to control group (15.02±0.19fl vs 14.12±0.22fl).

The P-LCR is not often mentioned in literature, probably because it is relatively a new platelet volume parameter. It is generated by only a few machines, with the Sysmex analyser being one of them.

Our study concluded that P-LCR were significantly higher ($p<0.005$) in diabetic patients. This is in agreement with the other studies done by Jindal, et al. [24]; Walinjkar, et al. [18] and Shilpi, et al. [19] that concluded significantly higher P-LCR in diabetic patients compared to non-diabetic individuals (Table 4).

Our study suggests that P-LCR (platelet index) needs careful observation in diabetic patients and must not be overlooked compared to common PDW and MPV indices. Jindal, et al. [24] carried out study among 75 subjects with DM (50 with one or more microvascular complications) and 50 non-selected patients from the hospital as controls. The P-LCR were all significantly higher in diabetic patients compared to the control subjects ($P<0.05$).

The higher values of MPV, PDW and P-LCR indicate that they serve as better risk indicator of initial vascular complications in diabetes mellitus patients and can be used as a simple and cost-effective tool to assess vascular events.

Table 2: Comparison of MPV with other studies

Publication	No. of diabetic subjects	MPV (fl)	Controls	MPV (fl)	'p' value
Present study (2024)	130	10.6	130	9.9	0.0001
Walinjkar, et al. (2019) [18]	125	11.32	125	8.56	0.0001
Shilpi, et al. (2018) [19]	280	11.3	280	9.0	<0.05
Kodiatte, et al. (2012) [17]	300	8.29	300	7.47	<0.001
Vadatti, et al. (2015) [20]	171	7.91	37	6.91	<0.0001

Table 3: Comparison of PDW with other studies

Publication	Cases	PDW (fl)	Controls	PDW (fl)	'p' value
Present study (2024)	130	12.3	130	11.1	0.0006
Alhadas, et al. (2016) [21]	100	17.8	100	17.5	<0.001
Demirtas, et al. (2015) [23]	307	16.4	187	15.4	<0.001
Jabeen, et al. (2013) [22]	170	15.02	92	14.12	0.003
Jindal, et al. (2011) [24]	75	18.14	50	15.34	0.006

Table 4: Comparison of P-LCR with other studies

Publication	Diabetics	P-LCR (%)	Non-diabetics	P-LCR (%)	'p' value
Present study (2024)	130	29.0	130	24.1	<0.005
Walinjkar, et al. (2019) [18]	125	43.0	125	34.0	0.0001
Shilpi, et al. (2018) [19]	280	35.0	280	23.0	0.002
Jindal, et al. (2011) [24]	75	42.3	50	37.0	0.004

Conclusion

Increased platelet volume indices contribute to the prothrombotic state in diabetes mellitus. Because larger platelets are hemostatically more active, its presence probably is a risk factor for developing diabetic vascular complications. Microvascular complications of diabetes are associated with higher platelet indices, as compared to diabetic patients without vascular complications.

The increased platelet activity and aggregation contribute to an increased risk of atherosclerosis and associated complications. Hence, increased MPV, PDW and P-LCR as the cause or the end result of vascular complications needs to be further explored.

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