THE NEW ARMENIAN MEDICAL JOURNAL
Vol.16 (2022), No 2 p.118-124
DOI: https://doi.org/10.56936/18290825-2022.16.2-118

EFFECTIVENESS OF PLATELET INDICES IN PREDICTING TYPE 2 DIABETES MELLITUS MICROVASCULAR COMPLICATIONS

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Received 12.10.2021; accepted for printing 30.05.2022

Abstract

Objective - This study was conducted to evaluate the efficacy of platelet indices, namely mean platelet volume, platelet distribution width, plateletcrit and platelet count for prediction of microvascular complications of type 2 diabetes mellitus.

Methods - A hospital-based, single-centre, observational, matched case-control study was conducted. A total of 120 diabetic cases and 120 non-diabetic controls were recruited and various biochemical parameters (fasting and post-prandial blood glucose, HbA1c, mean platelet volume, platelet distribution width, plateletcrit, platelet count and others) were measured. All diabetic cases were subjected for diagnosis of retinopathy, nephropathy or neuropathy. Statistical analyses were performed using unpaired t-test and Pearson's correlation test.

Results - The mean (±SD) age of the diabetic cases and controls were 59.8 (±11.2) years and 53.61 (±10.66) years, respectively. The overall male:female distribution was 64.2%:35.8% and 55.8%:44.2% in diabetic cases and controls, respectively. Platelet count and plateletcrit were found to be significantly higher in diabetic cases than controls (288741 (±97447)/µL vs. 255041 (±63883)/µL, p-value = 0.002; 0.234±0.072% vs. 0.201±0.043%, p-value = 0.0002). However, mean platelet volume and platelet distribution width in diabetic cases were not found to be significantly changed when compared to controls. Plateletcrit showed positive correlation with HbA1c with Pearson's correlation coefficient of r=0.19 (p-value <0.05). Mean platelet volume and platelet distribution width were found to be not-significantly correlated with HbA1c. Among diabetics, only plateletcrit was found to be significantly raised in cases with all complications compared to cases with no complication (p-value <0.05).

Conclusion - Plateletcrit and platelet count were found to be efficacious in forecasting the microvascular complications in type 2 diabetes.

Keywords: *diabetes mellitus, microvascular complication, plateletcrit, mean platelet volume, platelet distribution width, platelet count*

Introduction

"Diabetes Mellitus" is a group of common metabolic disorders that share the hyperglycemic phenotype" [*Kasper DL et al., 2018*]. The widespread prevalence and morbidity of diabetes are well known. According to the International Diabetes Federations, there are approximately 74 million cases of Diabetes Mellitus in India as of 2021, with a 9.6% age-adjusted comparative prevalence [*IDR-2021*]. Chronic hyperglycemia causes a number of distinct micro- and macro-vascular complications. Diabetic retinopathy, diabetic peripheral neuropathy, and diabetic kidney disease/nephropathy (DKD/DN) are examples of the former. Diabetes is one of the leading causes of adult blindness

Cite This Article as:
Ashwani K, Raghavendra R, Sujatha B; Effectiveness of platelet indices in predicting type 2 diabetes mellitus microvascular complications; NAMJ v.16 (2022) no.6, p. 118-124;
DOI: https://doi.org/10.56936/18290825-2022.16.2-118

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[*Kasper DL et al., 2018*]. Diabetic retinopathy is caused by retinal ischemia and hypoxia. The most common cause of chronic kidney disease, end-stage renal disease, and chronic kidney disease requiring renal replacement therapy is DKD/DN [*Kasper DL et al., 2018*]. Diabetic neuropathy affects nearly half of T2 Diabetes Mellitus patients and has a wide range of symptoms, patterns, and courses. Distal symmetric polyneuropathy is the most common type [*Kasper DL et al., 2018*].

Diabetes Mellitus is distinguished by a prothrombotic state of platelets caused by persistent hyperglycemia and insulin resistance, which causes pericytes and endothelial injury. It is believed that platelet activation contributes to the vascular problems of this metabolic disorder [*Koltai K et al., 2006*]. Platelets are critical to the integrity of normal homeostasis, and platelet indices (PIs) serve as an indicator of their function and morphological state [*Budak Y et al., 2016 Golebiewska E, Poole A, 2015*]. The relevant PIs, namely mean platelet volume, plateletcrit, and platelet distribution width, are easy to obtain and are widely available with routine blood counts. mean platelet volume represents an average platelet size associated with changes in either the rate of platelet production or platelet stimulation/activation. Platelet distribution width is a measure of platelet size distribution variation that indicates platelet anisocytosis. plateletcrit denotes the percentage of platelet volume in the blood.

The goal of the study was to find if a change in platelet indices (PIs) correlates with Diabetes Mellitus and its micro-vascular complications and such that to evaluate their role in predicting the micro-vascular complications. Diabetes Mellitus and its complications are major contributors to morbidity and economic burden. Correlating simple, inexpensive, readily available determinants to the likelihood of developing microvascular complications, will help with easing screening and follow-up of such patients. Therefore, we aimed to investigate the effectiveness of PIs in anticipating microvascular complications of type 2 Diabetes Mellitus.

Material and Methods

Study population: This was a hospital-based, single-centre, observational, matched case-control study conducted in Kasturba Medical College, Manipal from December 2020 to August 2021. The study protocol was approved from the Institutional Ethics Committee (IEC-87/2020) before the commencement of the study. Clinical Trials Registry India registration (CTRI/2020/12/030025) was done before beginning enrolment of subjects.

During the study period, 120 diabetic cases and 120 non-diabetic controls were collected. This number was arrived at by using the logistic regression-based formula, with a 0.5 baseline probability and 0.7 probability with predictors, for 80% power and 5% confidence interval.

Inclusion criteria: Individuals above the age of 18 years, diagnosed with type 2 Diabetes Mellitus, according to the criteria laid out by the American Diabetic Association [*ADA-2021*], with or without microvascular complications, were enrolled to serve as cases. The criteria for diagnosis of Diabetes Mellitus were established if haemoglobin A1c (HbA1c) ≥ 6.5% and/or casual blood glucose level of ≥200 mg/dL and/or fasting blood glucose level of ≥126 mg/dL and/or 2 hours postprandial blood glucose of ≥200 mg/dL during an oral glucose tolerance test. Age and Sex matched normal i.e., non-diabetic individuals (n=120) were recruited as controls who had a fasting blood glucose level of ≤110 mg/dL.

Exclusion criteria: Subjects with any malignancies, myeloproliferative disorders, infections, pregnancy, thrombocytopenia, hypothyroidism, and recent blood transfusion were excluded from the study. Subjects on drugs –such as anti-platelet agents and bone marrow suppressing drugs were also excluded from the study. Subjects having Type 2 Diabetes Mellitus with macrovascular

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

complications and severe illnesses were not participated.

Sample collection and laboratory investigations: The blood sample (amount - 5 ml) from cases and controls was collected in vacutainer with EDTA as an anti-coagulant and processed to obtain laboratory data including fasting blood glucose (FBG), postprandial blood glucose (PPBG), HbA1c, haemoglobin concentration, total leukocyte count, platelet count, mean platelet volume, plateletcrit and platelet distribution width, serum creatinine, serum potassium and thyroid stimulating hormone.

Characterization of diabetic retinopathy, DN, and diabetic peripheral neuropathy in type 2 diabetic cases: Subject details were obtained based on the use of a proforma, which included history and physical examination of the patient, along with relevant laboratory data.

For assessment of diabetic retinopathy, direct ophthalmoscopy, fundus fluorescein angiography, or optical coherence tomography, as carried out by the Department of Ophthalmology as a part of the routine evaluation on patients with diabetes, was used, if available. Else, based on symptomatology and bedside fundoscopic evaluation, patients were categorized as having diabetic retinopathy.

For categorization as DKD/DN, evaluation of albuminuria/proteinuria by Urine PCR or urine protein estimation in a random sample in the biochemistry lab was considered, with or without an elevated serum creatinine.

For categorization as diabetic peripheral neuropathy, clinical examination involving sensory perception checked by 10g monofilament, pain perception by pin-prick, vibration by a 128-hertz tuning fork, and/or nerve conduction velocity study, was used.

Statistical analysis and interpretation: SPSS software was used for statistical analysis (version 20). The mean ± standard deviation (SD) was used to express all numerical data. To compare the significant difference between the groups, an unpaired *t*-test was used, with a *p*-value of <0.05 considered significant. Pearson correlation was used to investigate the relationship between PIs and biochemical parameters.

Results

A total of 243 cases were selected for this study out of which 120 diabetics cases and 120 non-diabetics controls were enrolled in the study. Three patients were excluded in which 2 were detected to have malignancy and 1 was found to be an active smoker. The mean (±SD) age of the diabetic cases and controls were 59.8 (±11.2) years and 53.61 (±10.66) years, respectively. The overall male:female distribution was 64.2%:35.8% and 55.8%:44.2% in diabetic cases and controls, respectively. There were 38 cases of diabetic retinopathy, 41 with DKD, and 66 with diabetic peripheral neuropathy cases among the 120 cases. Thirty diabetic patients had no microvascular complications, while 12 patients had all three complications (Table 1).

The results of blood glucose parameters (FPG, PPBG and HbA1c) are shown in Table 2. The overall mean (±SD) values of FPG, PPBG and HbA1c in diabetic cases were 169.47 (±76.87) mg/dL, 237.43 (±97.27) mg/dL and 8.76

Table 1.

Baseline characteristics of study population

Demographic parameters	Cases	Control
Sample size, n	120	120
Male, n (%)	77 (64.2)	67 (55.8)
Female, n (%)	43 (35.8)	53 (44.2)
Male:Female ratio	1.79	1.26
Age, mean±SD (years)	59.8±11.2	53.61±10.66
Cases with DPN, n (%)	66 (55)	0
Cases with DR, n (%)	38 (31.7)	0
Cases with DKD, n (%)	41 (34.2)	0
Cases with no microvascular complications, (n)	30	0
Cases with 1 microvascular complication, (n)	47	0
Cases with 2 microvascular complications, (n)	31	0
Cases with all 3 microvascular complications, (n)	12	0
Total	120	0

Notes: DPN-diabetic peripheral neuropathy, DR -diabetic retinopathy, DKD - diabetic kidney disease

Table 2.

Data showing comparison of biochemical parameters and platelet indices among cases and healthy controls (Mean±SD)

Clinical Parameters	Cases	Control	*t*-test *p*-value (significance)
FBG (*mg/dL)*	169.47±76.87	100.75±9.81	<0.001 (***)
PPBG (*mg/dL*)	237.43±97.27	114.77±28.71	<0.001 (***)
HbA1c (%)	8.76±2.27	5.76±0.34	<0.001 (***)
Platelet indices			
Platelet count (*per μL*)	288741±97447	255041±63883	0.002 (**)
MPV (fl)	8.08±0.97	8.09±1.07	0.939 (ns)
PCT (%)	0.234±0.072	0.201±0.043	0.0002 (***)
PDW (fl)	16.6±1.15	16.785±0.54	0.113 (ns)

Notes: *FBG - fasting blood glucose, PPBG - postprandial blood glucose, HbA1c - Hemoglobin A1c, MPV - mean platelet volume, PCT - plateletcrit, PDW - platelet distribution width*

(±2.27)%, respectively, which were found to be elevated with statistical significance (p<0.001) from those values of 100.75 (±9.81) mg/dL, 114.77 (±28.71) mg/dL and 5.76 (±0.34)% in non-diabetic controls.

The mean (±SD) platelet counts of 288741 (±97447)/μL observed in diabetic cases was found to be significantly higher from that of 255041 (±63883)/μL in non-diabetic controls (*p*-value = 0.002). The mean (±SD) values of plateletcrit in diabetic cases and controls were found to be 0.234±0.072% and 0.201±0.043%, respectively. There was a statistical significance (*p*-value = 0.0002) observed between diabetic cases and controls for the mean values of plateletcrit. The mean values of mean platelet volume and platelet distribution width were not significantly raised in diabetic cases when compared to non-diabetic controls (Table 2).

The PIs (mean platelet volume, plateletcrit and platelet distribution width) were correlated with biochemical parameter (HbA1c) in diabetic cases to study any association between these markers. Only plateletcrit values showed positive correlation with HbA1c with Pearson's correlation coefficient of r=0.19 with a *p*-value of <0.05. The other indices, mean platelet volume and platelet distribution width were found to be not-significantly correlating with HbA1c (Table 3).

Sub-group analysis was carried out by comparing PIs (mean platelet volume, plateletcrit, platelet distribution width) among diabetic cases with any complication, with all complication, and with no complications. Comparing mean platelet volume values in cases with any complication, with all complication, and no complications were comparable and remained statistically non-significant. The mean (±SD) plateletcrit values were found to be significantly raised in cases with all complication, 0.221 (±0.07)% when compared to cases with no complication, 0.208 (±0.055)% with *p*-value of <0.05. The mean (±SD) platelet distribution width values were significantly higher in cases with no complication, 16.77 (±0.53) fl than cases with all complications, 16.08 (±2.15) fl with *p*-value of <0.05. The mean HbA1c in diabetic

Table 3.

Correlation of hba1c with platelet indices (mpv, pct, pdw)

Clinical parameter		HbA1c (%)
MPV (fl)	Correlation coefficient (r)	0.05
	p-value	0.436
PCT (%)	Correlation coefficient (r)	0.19
	p-value	<0.05
PDW (fl)	Correlation coefficient (r)	0.006
	p-value	0.91

Notes: *MPV - mean platelet volume, PCT - plateletcrit, PDW - platelet distribution width*

Table 4.

Comparison of platelet indices (MPV, PCT, PDW) among diabetic cases with any complication, with all complication, and no complications (Mean±SD)

Platelet indices	Any complication (n=90) [A]	With all complication (n=12) [B]	No complication (n=30) [C]	t-test (p-value, significance) A vs. C	B vs. C
MPV (fl)	8.20±1.01	8.22±1.53	8.02±1.03	0.19, ns	0.537, ns
PCT (%)	0.232±0.07	0.221±0.07	0.208±0.055	<0.05, **	0.443, ns
PDW (fl)	16.55±1.29	16.08±2.15	16.77±0.53	0.07, ns	<0.05, **

Notes: *MPV - mean platelet volume, PCT - plateletcrit, PDW - platelet distribution width*

cases with no complications and cases with all complications were found to be 6.26 and 8.92%, respectively (Table 4).

Discussion

Diabetes, whether type 1 or type 2, makes a patient prone to a number of complications, which can be classified into microvascular, macrovascular, and other complications. Diabetes is identified as a condition caused by altered hepatic glucose metabolism, impaired insulin action, reduced insulin secretion, aberrant lipid and muscle metabolism, and low-grade systemic inflammation. There are numerous proposed mechanisms underlying these effects, and no one explanation has yet been definitively supported.

Diabetes often results into its vascular complications classified as microvascular and macrovascular. The former complications are due to two important factors: the duration of diabetes and optimal glycemic control, or lack thereof. These diseases place a significant burden on the healthcare system and must be addressed with urgency. Diabetes is a pro-inflammatory state predisposed to systemic inflammation, which allows for the use of specific inflammatory markers to monitor the activity and progression of the disease and its complications. Platelets have also been shown to play a role in the inflammatory cascade [*Morrell C et al., 2014; Repsold L, Joubert A, 2021*]. As a result, it was attempted to determine whether PIs can serve as a predictor of diabetes progression in the form of the onset of microvascular complications. Therefore, the purpose of this study was to determine the role of mean platelet volume, plateletcrit, and platelet distribution width, all of which are readily available PIs, in envisaging the microvascular complications of type 2 diabetes. PIs were compared in this study between cases with type 2 Diabetes Mellitus and non-diabetic healthy controls. In our study, it was found that plateletcrit levels in the cases were found to be substantially greater than in the controls. For mean platelet volume and platelet distribution width, there were no statistically significant variations between the two groups.

HbA1c, a glycemic control indicator, was also found to have a positive and statistically significant relationship with plateletcrit. Although there was a positive correlation between mean platelet volume and HbA1c, it was not statistically significant. Platelet distribution width, on the other hand, had a negative correlation with glycated haemoglobin. In contrast, Walinjkar et al. found that diabetics had substantially higher mean platelet volume, platelet distribution width, P-LCR, and plateletcrit when compared to non-diabetics. Additionally, it was discovered that patients with microvascular problems experienced greater increases in mean platelet volume, platelet distribution width, and P-LCR than patients without these issues [*Repsold L, Joubert A, 2021*]. This discrepancy in the outcome may be a result of unique patient profiles of the study. Additionally, individuals with diabetes who also had ischemic heart disease and were on anti-platelet medications, which would have impacted platelet function, were not taken into account or included. Akinbami et al. investigated the “correlation between mean platelet volume and platelet count among patients with and without diabetes and discovered that mean platelet volume was not significantly different between the two groups” [*Walinjkar R et al., 2019*]. Moreover, our study discovered a statistically significant rise in platelet count when contrasting cases with controls. Our investigation also did not find any statistically significant difference in mean platelet volume between cases and controls. This is consistent with the literature’s assertion that, under physiological settings, platelet count and mean platelet volume are negatively associated in order to maintain homeostasis [*Akinsegun A et al., 2014; Zareifar S et al., 2014*].

To ascertain whether the PIs associated with the development of diabetes and the extent of present microvascular problems, research on the platelet determinants was also conducted. We examined diabetes with no microvascular complications, diabetes with one or more microvascular difficulties, and diabetes with all three microvascular complications for these analyses. Our research found no signifying results for mean platelet volume or platelet distribution width, but did find substantial elevation for plateletcrit in the group with any mi-

crovascular problem when compared to cases with no vascular complications. platelet distribution width, but not plateletcrit, demonstrated statistical significance in being lower among individuals who had all three microvascular problems when compared to those who did not have any issues. In any of the analyses, mean platelet volume did not show a significant correlation.

Cases had HbA1c levels considerably higher than in the controls, as expected (8.76% *vs.* 5.6%). In subgroup analysis, it was discovered that diabetics who had additionally experienced any of the microvascular problems had HbA1c levels that were considerably higher (8.93% *vs.* 8.2%). When the numbers were contrasted between individuals with no microvascular problems and those with all three microvascular issues, the difference was much more obvious (9.14% *vs.* 8.28%). As a result, this study supports the notion that with the rise of HbA1c levels, the patient's risk of microvascular complications rises. Elevated readings are also sign of poorly managed diabetes, which puts patients at an increased risk of developing complications. The readings of glycated haemoglobin were found to be lower in groups that included DKD patients than in groups that did not include DKD patients but had other microvascular problems. Due to the anaemia of chronic disease that DKD patients experience, this finding can be puzzling.

The background of numerous studies provides the knowledge that HbA1c can aid in monitoring and treatment decisions. The "ADVANCE" trial in particular showed that risk of death, microvascular complications, or macrovascular complications all increased significantly above certain threshold values of glycated haemoglobin, namely >7% for macrovascular complication and death, and >6.5% for microvascular complications, with rises in risk of 38 percent, 40 percent, and 38 percent, respectively [*Zoungas S et al., 2012*]. Patients with elevated mean HbA1c had a considerably larger risk of cardiovascular mortality and "time-to-death," according to Chinese research led by Lee et al [*Lee S et al., 2021*]. Eliminating the underestimation of how important HbA1c can be in monitoring Diabetes Mellitus patients would require an "updated mean of HbA1c", according to a Swedish study by Lind et al., who also recommends using this measure instead of baseline glycated haemoglobin readings. It was also said that it did not illustrate the "time-dependent effect of HbA1c" [*Lind M et al., 2009*].

Platelets are involved in many disorders since they are a part of the inflammatory process. When assessing the importance of PIs for a particular condition, we should be mindful that there may be numerous possible variables. Several medicines and the majority of the frequent causes of this have been left out of this study. This study attempted to assess the function of PIs in anticipating microvascular complications of type 2 Diabetes Mellitus, with encouraging results despite the small sample size of 240 patients.

Conclusion

This study found a link between plateletcrit and the development of microvascular problems, which is a sign of Diabetes Mellitus progression. Additionally, it demonstrated a strong correlation with cases, or Diabetes Mellitus patients as opposed to non-diabetics. Therefore, it could be used to foretell Diabetes Mellitus microvascular problems.

In individuals with Diabetes Mellitus, platelet distribution width was similarly observed to decline as complications increased. Plateletcrit and higher HbA1c levels associated well. Additionally, it revealed a strong association between Diabetes Mellitus and the occurrence of microvascular problems. Further research is necessary because there is conflicting information about the ability of mean platelet volume and platelet distribution width to predict these problems in Diabetes Mellitus.

Acknowledgments: *Ashwani Kumar contributed to the background literature review, data acquisition and manuscript preparation. Sujatha Bagepalli contributed to manuscript preparation, design of the study and data analysis. Raghavendra Rao contributed to the conception and design of the study, background literature review, statistical analysis and revision of the manuscript.*

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Vol.16 (2022). No 2

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THE NEW ARMENIAN MEDICAL JOURNAL

Vol.16 (2022). No 2

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Our journal is registered in the databases of Scopus, EBSCO and Thomson Reuters (in the registration process)

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Copy editor: Tatevik R. Movsisyan

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