

## Research Article

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# Study of altered platelet morphology with changes in glycaemic status

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## ABSTRACT

**Background:** Diabetes is a pandemic causing very high morbidity and mortality due to its complications which are a result of micro and macro angiopathy. Platelets play a key role in the vascular complications. These complications are attributed to platelet activation which can be recognised by an increase in platelet volume indices (PVI) including mean platelet volume (MPV) and platelet distribution width (PDW). Platelet indices can be potentially useful surrogate markers for the early diagnosis of thromboembolic and cardiovascular complications in diabetes.

**Methods:** This is a cross-sectional study conducted for 2 years with total 930 subjects. The patients were segregated in 03 groups on basis of HbA1C as (a) Diabetic, (b) Non-Diabetic and (c) IFG. Samples for HbA1C and platelet indices were collected using EDTA (ethylene diamine tetracetic acid) as anticoagulant and were processed on autoanalysers.

**Results:** The study revealed a stepwise increase in the PVI from non-diabetics to IFG to diabetics. MPV and PDW were increased in the IFG cases as compared to the non-diabetic and were markedly increased in the diabetic patients. MPV and PDW of diabetics, IFG and non-diabetics were  $17.60 \pm 2.04$ ,  $11.76 \pm 0.73$ ,  $9.93 \pm 0.64$  and  $19.17 \pm 1.48$ ,  $15.49 \pm 0.67$ ,  $10.59 \pm 0.67$  respectively with a significant p value 0.00. Significant positive correlation between PVI with glycaemic levels and duration of diabetes across the groups (MPV-HbA1c r = 0.951; PDW-HbA1c r = 0.875). However, the total platelet count was found to decrease with the increasing glycaemic levels with a p value <0.001. A significant negative correlation was found between glycaemic levels and total platelet count (PC- HbA1c r = -0.164).

**Conclusions:** This study showed that platelet morphology is altered with increasing glycaemic levels. These changes can be known by measurements of PVI which is an important simple and effortless tool can be used more extensively to predict the acute vascular events and thereby help curb morbidity and mortality.

**Keywords:** MPV, PDW, PVI, IFG, HbA1C, Diabetes

## INTRODUCTION

Diabetes mellitus (DM) is not a single disease entity but a group of metabolic disorders which share the common underlying features of hyperglycaemia resulting from interactions between environmental factors and polygenic inheritance. It is characterised by metabolic abnormalities, chronic hyperglycaemia and long term macro vascular & micro vascular complications involving blood vessels, nerves, eyes and kidneys.<sup>1,2</sup> Approximately 382 million people are suffering from diabetes worldwide

and this number is expected to increase to 592 million by 2035. India ranks first in the world in having the largest number of absolute diabetics i.e. 19 million followed by China and US.<sup>3</sup>

The development of diabetic angiopathies is preceded by activated platelets which play an important role in its pathogenesis.<sup>4</sup> Both DM type I and type II have platelet hyperactivity, dysfunction and altered morphology which leads to thrombus formation, microvascular embolisation and release of constrictive, oxidative and mitogenic

substances and play a key role in acute coronary events and other thromboembolic diseases.<sup>5</sup>

These alterations in platelet morphology, activation and function can be known by the platelet volume indices (PVI) which includes mean platelet volume (MPV) and platelet distribution width (PDW). MPV and PDW can be used as a marker for early diagnosis of diabetic complications as a large proportion of patients with DM type II suffer from preventable vascular angiopathies. This is an important, simple, effortless and cost effective tool which should be used and explored especially in developing countries like India for predicting the upcoming acute vascular events and preventing them by risk factor modifications and treatment.

The aim of the study was to evaluate and compare the platelet indices in the patients with type II DM, IFG and non-diabetics, to evaluate the correlation of platelet indices with blood glucose regulation and duration of diabetes & to determine the association between PVI and vascular complications and their utility in early recognition of such complications.

## METHODS

This is a cross-sectional study. Patient attending the outpatients and inpatients department of INDEX Hospital were included during study period of 2 years from June 2012 to May 2014. Appropriate prior consent was taken from the patients and clinical details were recorded in the proforma.

All the investigations were done in the pathology and clinical biochemistry laboratory of INDEX Hospital, Indore.

Samples for platelet volume indices and HbA<sub>1C</sub> were collected using EDTA as anticoagulant and processed on a SYSMEX X-800i and Erba EM 360 autoanalysers.

**Table 1: Criteria for the diagnosis of diabetes.**

<b>A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*</b>		
OR		
	MPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hour.*	
OR		
	Performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*	
OR		
	In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).	
*In the absence of unequivocal hyperglycemia, result should be confirmed by repeat testing.		

3 groups were formed on the basis of glycaemic control - diabetic, non-diabetic and impaired fasting glucose group respectively. The ADA criteria was used for segregation of patients in 3 groups.<sup>6</sup>

**Table 2: Criteria for the diagnosis of diabetes (prediabetes)\*.**

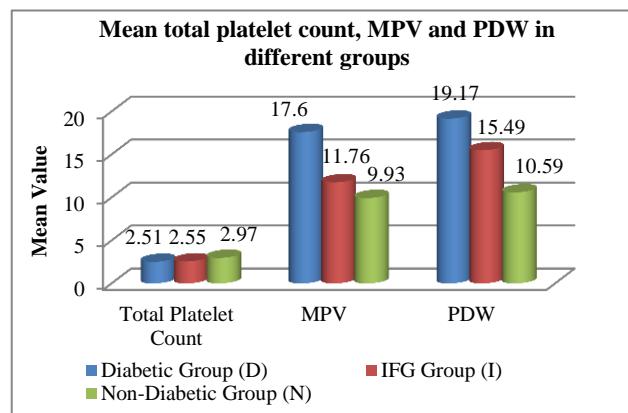
FPG 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (IFG)
OR
2-h plasma glucose in the 75-gOGTT 140 mg/Dl (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) (IGT)
OR
A1C 5.7–6.4%
*For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at higher ends of the range.

Controls (Non diabetics) were those with HbA<sub>1C</sub> <5.7% were put under non-diabetic group. The diabetic group comprised of cases with HbA<sub>1C</sub> ≥6.5% and those with HbA<sub>1C</sub> between 5.7% and 6.4% were put under IFG group.

The MPV and PDW of all three groups was compared, studied and analysed. Patients suffering from following diseases viz idiopathic thrombocytopenic purpura, iron deficiency anaemia, acute post streptococcal glomerulonephritis, renal failure, myocardial infarction, ischaemic heart disease and stroke, cyanotic congenital heart diseases, hypertension were excluded.

The obtained data was tabulated using MS Excel to create a master chart. The power of study was kept at 99% and level of significance ( $\alpha$ ) at 5%. Statistical analysis was done by the statistical package for social sciences (SPSS) version 16 for windows.

## RESULTS



**Figure 1: Comparison of the glycaemic characteristics (FPG & HbA<sub>1C</sub>), platelet counts and platelet volume indices (MPV & PDW) of study participants in impaired fasting group (I) and the non diabetic (D).**

A total of 1100 patients were evaluated, out of which 930 fulfilling the inclusion criteria were selected and allocated 3 groups according to their glycaemic status.

A stepwise increase in the platelet volume indices was found (Table 3) (Figure 1).

**Table 3: Comparison of the glycaemic characteristics (FPG & HbA1c), platelet counts and platelet volume indices (MPV & PDW) using ANOVA (analysis of variance).**

Parameter	Diabetic group (D) (n=330)	IFG group (I) (n=300)	Non-diabetic group (N) (n=300)	F value	P value	Remark
FPG (mean ± SD) (fl)	128.12±37.26	109.36±7.81	98.09±4.26	139.23	<0.001	S
HbA <sub>1c</sub> (mean±SD) (%)	9.55±1.80	6.01±0.21	4.97±0.32	1521.09	<0.001	S
TotalPlatelet count (mean±SD) (lacs)	2.51±0.69	2.55±0.71	2.97±0.82	34.56	<0.001	S
MPV (mean±SD) (fl)	17.60±2.04	11.76±0.73	9.93±0.64	2832.89	<0.001	S
PDW (mean±SD) (μm)	19.17±1.48	15.49±0.67	10.59±0.67	5345.21	<0.001	S

## DISCUSSION

DM is a strong independent predictor of short and long term recurrent ischaemic events. Patients with diabetes mellitus (DM) have a higher risk of complications and recurrent antithrombotic events which worsens the prognosis of vascular angiopathies as compared to non diabetic patients.<sup>7</sup> Both macro and micro vascular complications occur in DM and includes coronary artery disease (CAD), atherosclerosis, medial calcification, retinopathy, nephropathy, neuropathy and peripheral arterial disease (PAD).<sup>8</sup> Data reveals that in DM there is a 2-4 fold increase in CAD and PAD and a 3 fold increase in stroke as compared to non diabetic subjects.

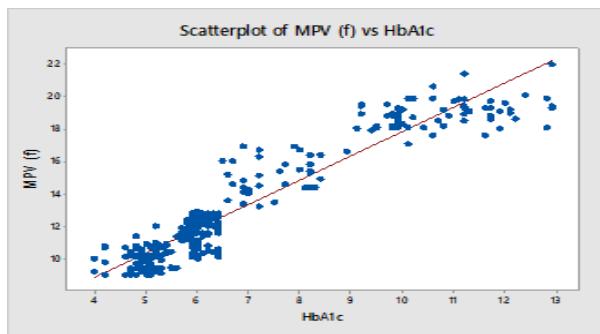
DM is considered as a prothrombotic state characterised by increased platelet activation and coagulation & decreased fibrinolytic activity.<sup>9</sup> In diabetes there is an alteration in all the systems that maintain the integrity and patency of the blood vessels and a vicious circle of events is initiated in the vascular wall which includes platelet hyperactivity and dysfunction, increased inflammation, altered coagulation and endothelial dysfunction.<sup>10</sup>

Previous studies have shown that in DM there is increased thrombopoiesis and circulation of large, young and activated platelets which undergo more frequent episodes of release of granules, accelerated sequestration in the circulation and have reduced survival.<sup>4</sup> The alteration of platelets in diabetes are increase in its size and hyperactivity and these changes are proposed to be caused due to hyperglycaemia, insulin deficiency and resistance, associated metabolic conditions and other cellular abnormalities including endothelial dysfunction, oxidative stress and inflammatory state.<sup>11</sup> These altered platelet function and activity cause thrombus formation,

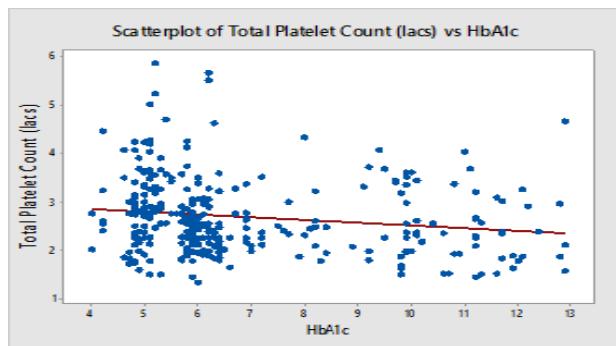
micropapillary embolisation and release of constrictive, oxidative and mitogenic substances which further accelerates the progression of local vascular lesions.<sup>9</sup> Activated platelets also release platelet microparticles which contain platelet adhesive molecule, proinflammatory factors and cause inflammatory reactions.<sup>12</sup> These platelets are metabolically and enzymatically more active than the smaller ones & are more reactive containing more granules and produce more thromboxane A2, beta-thromboglobulin, serotonin & secrete more membrane receptors such as CD63 and CD62 leading to more thrombotic adhesion and aggregation.<sup>2</sup>

MPV is a marker of platelet function, activity, size and is directly proportional to platelet aggregation i.e. higher the MPV, more is the platelet aggregation in response to collagen and ADP. MPV also depends on the platelet granule content of various platelet specific proteins, number of glycoprotein molecules on platelet membrane and the thromboxane synthesising capacity.<sup>4</sup> Changes in MPV reflects the state of thrombogenesis. A procoagulant effect is generated by increased MPV which causes thrombotic vascular complications.<sup>13</sup> PDW is affected by the increased number and size of pseudopodia.

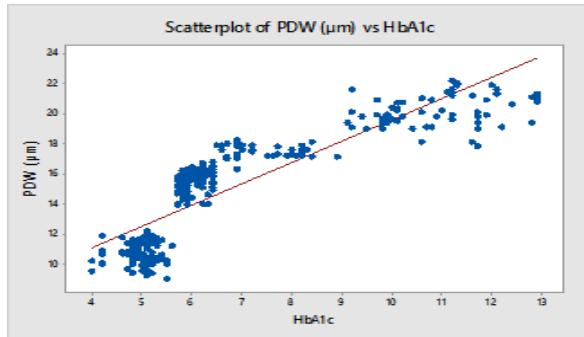
The present study observed a gradual increase in PVI in non-diabetics, IFG and diabetics. MPV and PDW were found to be the highest in Diabetics. PVI were also increased in patients with impaired fasting glucose as compared to the non diabetics. A linear correlation was found between PVI and the glycaemic levels. Also a direct relationship was observed between PVI and duration of diabetes as depicted by the scatter plots below (Figure 2a-2f).



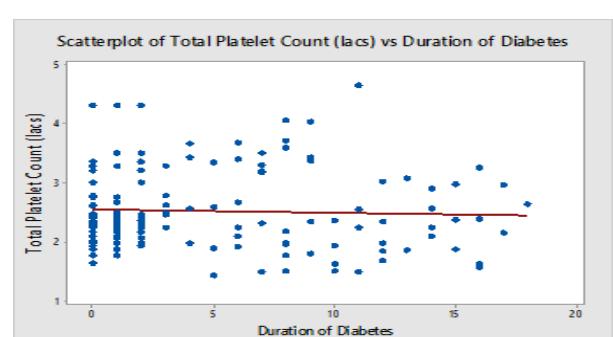
**Figure 2a:** Scatter plot showing a positive correlation between MPV and HbA1c.



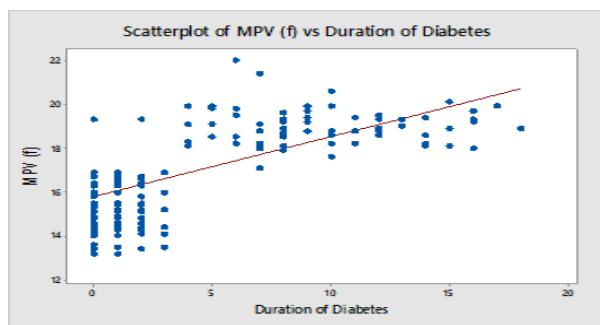
**Figure 2e:** Scatter plot showing a positive correlation between total platelet count and HbA1c.



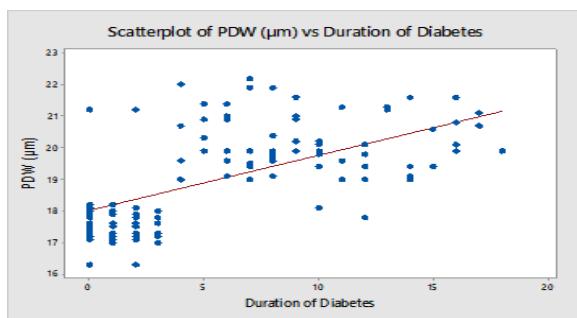
**Figure 2b:** Scatter plot showing a positive correlation between PDW and HbA1c.



**Figure 2f:** Scatter plot showing a positive correlation between total platelet count and duration of diabetes.



**Figure 2c:** Scatter plot showing a positive correlation between MPV and duration of diabetes.



**Figure 2d:** Scatter plot showing a positive correlation between PDW and duration of diabetes.

These findings were similar to the study by Zuberi B, et al which reported the increase in MPV in diabetics and IFG with respect to non diabetics.<sup>8</sup> E Vagdatli, et al noted that MPV and especially PDW increase during platelet activation and PDW is a more specific indicator.<sup>14</sup> Shimodaira, et al confirmed a direct relationship between MPV and Fasting blood glucose.<sup>15</sup> Kemal T Ulutas, et al confirmed a direct relationship between MPV and blood glucose regulation.<sup>4</sup> Thomax Alex Kodiatt, et al also documented that MPV was significantly higher in diabetics than in non diabetics.<sup>11</sup>

This study an inverse relationship between total platelet counts and glycaemic levels. The platelet count was slightly decreased in the diabetic group as compared to the IFG and the non diabetic group. The highest platelet count was seen in the non diabetic group and lowest in the diabetic group. These findings were in agreement with previous studies like Shimodaira, et al Kir Young Kim, et al etc which also reported a decrease in platelet count with increased glycaemic levels.<sup>15,16</sup>

In this study we have shown a significant stepwise increase in PVI from a non diabetic population to IFG to a diabetic population. Diabetic patients are known to have higher incidence of stroke and myocardial infarction. PVI can be used as a risk marker in Type II DM. Presence of higher MPV and PDW in these patients is an important finding that could increase the risk of thrombotic complications. Another important finding in

the study was the presence of significantly higher PVI values in IFG patients as compared to non diabetics. This shows that IFG's are also at increased risk of the vascular complications.

## CONCLUSION

This study showed that platelet morphology is altered with increasing glycaemic levels. These changes can be known by measurements of PVI which is an important simple and effortless tool can be used more extensively to predict the acute vascular events and thereby help curb morbidity and mortality.

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*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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