Original Article

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An Evaluation Study of Platelet Volume Indices (PVI) in Type-2 Diabetes Mellitus and its Micro and Macro Vascular Complications.

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ABSTRACT

Background: The study was performed to analyse PVI such as mean platelet volume (MPV), platelet distribution width (PDW) and platelet-large cell ratio (P-LCR) that are useful for identifying large and haemostatically active platelets, which are risk factors for developing diabetes and its complication.

Methods: Case–control study was conducted on 1026 Type 2 diabetics and 616 nondiabetics. Detailed clinical history regarding duration, hypertension and complications was taken. PVI was obtained using three part automated cell counter. Fasting blood glucose, hemoglobin A1C, lipid profile, creatinine were also obtained. Diabetics were further categorized into patients with complications and without complications.

Result: MPV, PDW, P-LCR and platelet count were significantly increased in diabetic patients with complications as compared to diabetics without complications and nondiabetic group ($P < 0.0001$, < 0.0001, 0.0044, 0.023 respectively). We found statistically significant correlation between MPV and diabetic retinopathy ($P < 0.0001$), nephropathy ($P = 0.04$), neuropathy ($P < 0.0001$), coronary artery disease ($P < 0.0001$), diabetic foot ($P = 0.005$). PDW was statistically significantly increased in diabetic retinopathy ($P < 0.0001$), neuropathy ($P < 0.0001$), coronary artery disease ($P < 0.0001$), diabetic foot ($P = 0.005$). P-LCR was statistically significantly increased in diabetic retinopathy ($P < 0.0001$), neuropathy ($P = 0.003$), coronary artery disease ($P < 0.0001$), diabetic foot ($P = 0.034$).

Conclusion: MPV, PDW and P-LCR are predictive biomarkers of diabetic vascular complications. They are more significant in microvascular complications than macrovascular complications.

Keywords: *Mean Platelet Volume, Platelet Distribution Width, Platelet-large Cell Ratio, Platelet Volume Indices, Diabetes Mellitus*

Introduction

Diabetes mellitus (DM) is a complex disease characterized by chronic hyperglycemia, metabolic abnormalities, and long-term macro- and micro-vascular complications involving the blood vessels, eyes, kidneys, and nerves.[1] Type 2 diabetes accounts for over 80% of cases of DM and is a slow onset, heterogeneous disorder resulting from interactions between environmental factors and polygenetic inheritance.[2]

Diabetic complications are mainly due to hyperglycemia and are responsible for the majority of morbidity and mortality associated with DM. Fasting blood sugar (FBS), postprandial blood glucose, and hemoglobin A1c (HbA1c) are widely used to monitor glycometabolic control in patients with DM. HbA1c is a more useful marker to determine mean blood glucose levels over a long time period. [3]

DM is considered as a "prothrombotic state" owing to sustained hyperglycemia, dyslipidemia, and insulin resistance causing endothelial and pericyte injury. Altered platelet morphology and function has been observed in diabetes in the form of enhanced platelet activity which may contribute to this "prothrombotic state". [4] Larger platelets that contain denser granules are metabolically and enzymatically more active than smaller ones and have higher thrombotic potential. Hence, increased mean platelet volume (MPV) and platelet distribution width (PDW) might be linked with increased thrombotic potential. [5]

The association of increased MPV, PDW, P-LCR, and platelet count with diseases related to endothelial dysfunction such as metabolic syndrome, diabetes, coronary artery disease (CAD), and malignancy has been shown in many studies. [6, 7, 8, 9]

The newer hematological analyzers are giving variety of platelet parameters which helps in easy detection of change in platelet structure, which may help in early detection of prothrombotic state of the platelets. These can act as an alarm for diagnosing initiation/progression of diabetic complications. Hence, in view of this, we aim to study platelet parameters in type 2 diabetes and its predictive role in diabetic angiopathies.

Materials and Methods

A prospective tertiary care hospital outpatient department based study was carried out on 1026 diabetic patients and 616 nondiabetic without CAD from 08/01/2016 to 31/10/2017. Institutional Ethical Committee clearance was obtained before the start of study. Informed and written consent of the patients was taken.

Three groups were studied. Group A: diabetic patients diagnosed in accordance with the American Diabetes Association with micro- and macro-vascular complications. [10] (386 patients); Group B: diabetic patients diagnosed in accordance with the American Diabetes Association without micro- and macro-vascular complications. [10] (640 patients); and Group C: healthy controls from health check-up without DM, as obtained from their medical records. Females with Hb <10 g% and males Hb <12 g%, nondiabetic subjects with CAD, pregnant women, patients on antiplatelet drugs such as aspirin and clopidogrel and subjects with any diagnosed malignancy were excluded from the study. (616 patients).

We used the histograms which were preserved in our clinical and hematological laboratory after running samples. 2 ml of blood was collected in dipotassium EDTA, fluoride and plain tubes each from all the patients. The dipotassium EDTA sample was run within two hours of venipuncture using the Biote 3215 automated three part cell counter in which Internal and external quality controls (Bio red 2 level : normal and high level control) with AIIMS EQAS participation were strictly followed. Plasma glucose levels were measured by the glucose oxidase method by fully automated biochemistry analyzer TC 6060 in which Internal and external quality controls (Agappe 2 level: normal and high level control) with CMC Vellore EQAS participation were strictly followed. HbA1c level was analyzed by immunoturbidometric inhibition method by RX 50 semi-automated biochemistry analyzer. Total cholesterol, triglyceride (TG), and high density lipoprotein (HDL) was measured by commercial enzymatic calorimetric method and low density lipoprotein (LDL) by direct enzymatic method.

In addition, diabetic patients were also evaluated regarding various microvascular complications such as diabetic retinopathy, neuropathy, and nephropathy, and macrovascular complications such as CAD, peripheral arterial disease (PAD), and diabetic foot. CAD findings were based on the clinical symptoms, 2D echocardiography (ECHO) observations of the patients. PAD had been diagnosed on the basis of clinical symptoms, ability to walk distances, and Doppler ECHO study of lower limbs. Diabetic foot was diagnosed based on five criteria of the National Diabetes Education Program: Lack of protective sensation (sensory neuropathy), absent pedal pulses, foot deformity, current or past foot ulcer, and history of foot amputation. [11]

Retinopathy diagnosis was based on the findings of at least two microaneurysms and/or retinal damage in the records. The quantitative urine albumin/creatinine ratio in the morning spot urine samples, increased BUN and creatinine was used for standard diagnosis of diabetic nephropathy. Diabetic neuropathy was based on the clinical records available.

The obtained parameters were evaluated using descriptive statistical analysis. Statistical analyses were performed using the IBM SPSS (Statistical Package for the Social Sciences v15.0) and Microsoft Excel 2007 software. The Student's *t*- test and the one way analysis of variance test were used for comparing the group means. The $\chi 2$ test was used and p values of <0.05 were taken as significant.

Result

A total of 1026 diabetic patients (521 males, 505 females) and 616 controls (314 males, 302 females) were selected for the study. Maximum number of diabetic males was in the age group of 50–68 years. Comparison of platelet count and platelet indices was made in cases and controls. The study suggested that MPV, PDW, P-LCR and Platelet count was significantly increased in diabetic patients with complications than diabetics without complications than nondiabetic group ($P < 0.0001$, < 0.0001, 0.0044, 0.023 respectively). [Table 1] [Figure 1]. The study reinforced the fact that poor glycemic control and raised FBS causes increased the risk of diabetic complications [Table 2]. The association of platelet indices with various diabetic complications was shown in [Table 3]. We found statistically significant correlation between MPV and diabetic retinopathy ($P < 0.0001$), nephropathy ($P = 0.04$), neuropathy ($P < 0.0001$), coronary artery disease ($P < 0.0001$), diabetic foot ($P = 0.005$) and in peripheral vascular disease, MPV is increased but not statistically significant. PDW was statistically significantly increased in diabetic retinopathy ($P < 0.0001$), neuropathy ($P < 0.0001$), coronary artery disease ($P < 0.0001$), diabetic foot ($P = 0.005$) and it is statistically insignificant in rest of the complications. P-LCR was statistically significantly increased in diabetic retinopathy ($P < 0.0001$), neuropathy ($P = 0.003$), coronary artery disease ($P < 0.0001$), diabetic foot ($P = 0.034$) and it is statistically insignificant in rest of the complications.

Table 1: Comparison of platelet volume indices in diabetics with complications, without complications and non-diabetics.

Group	Group A (n=386)	Group B (n=640)	Group C (n=616)	p Value
PC (×10^9/l)	180.16 (64.10)	220.20 (67.30)	220.90 (68.00)	0.023*
MPV (fL)	11.80 (1.20)	10.60 (0.96)	8.53 (0.93)	<0.0001*
PDW (fL)	14.60 (2.40)	13.20 (2.00)	11.34 (1.98)	<0.0001*
P-LCR (%)	28.71 (6.80)	24.32 (6.30)	20.23 (5.54)	0.0044*

*Values are mean (SD), * Significant p value, MPV- mean platelet volume (8-12fl); PC- platelet count (1.5-4.5 x 10^3/uL); PDW- platelet distribution width (9-14fl); P-LCR- platelet large cell ratio (11.9-45 %)*

Table 2: Comparison of FBS, HBA1c according to complications in diabetic group.

Complication	Yes/No	N	FBS (mg/dl)	p Value	HbA1c (%)	p Value
Diabetic retinopathy	Yes	386	183.37 (50.12)	0.70	10.6 (1.72)	<0.0001*
	No	640	160.40 (52.28)		8.5 (1.83)	
Nephropathy	Yes	220	172.20 (64.19)	0.72	9.47 (1.47)	0.06
	No	806	168.40 (60.22)		8.6 (1.81)	
Neuropathy	Yes	216	182.57 (49.16)	0.20	8.90 (1.90)	0.80
	No	810	164.11 (52.38)		8.70 (1.64)	
Coronary artery disease	Yes	312	190.19 (60.78)	0.010*	9.20 (1.92)	0.030*
	No	714	166.37 (60.32)		8.60 (1.68)	
Peripheral vascular disease	Yes	109	194.80 (64.81)	0.30	9.80 (1.94)	0.32
	No	917	172.30 (48.14)		8.70 (1.69)	
Diabetic foot	Yes	111	174.52 (58.66)	0.78	9.30 (1.99)	0.58
	No	915	170.48 (54.18)		8.80 (1.80)	

*Values are mean (SD), * Significant p value, FBS- Fasting blood sugar (70 to 100 mg/dl); HbA1c- hemoglobin A1c (4-5.6%), N= Number*

Table 3: Comparison of MPV, PDW, P-LCR according to the complications in diabetic group.

Complication	Yes/No	N	MPV (fL)	p Value	PDW (fL)	p Value	P-LCR (%)	p Value
Diabetic retinopathy	Yes	386	12.90 (2.21)	<0.0001*	15.10 (4.14)	<0.0001*	29.71 (9.80)	<0.0001*
	No	640	10.50 (1.98)		13.90 (4.10)		24.32 (10.20)	
Nephropathy	Yes	220	11.00 (2.32)	0.04*	13.97 (3.98)	0.21	26.30 (8.60)	0.57
	No	806	10.00 (1.97)		13.60 (4.1)		25.15 (10.00)	
Neuropathy	Yes	216	11.80 (1.98)	<0.0001*	14.98 (3.60)	<0.0001*	28.82 (10.20)	0.003*
	No	810	10.20 (2.10)		13.90 (4.00)		24.10 (8.90)	
Coronary artery disease	Yes	312	11.70 (1.67)	<0.0001*	14.91 (4.24)	<0.0001*	28.90 (14.20)	<0.0001*
	No	714	9.98 (1.50)		13.80 (3.98)		24.33 (10.30)	
Peripheral vascular disease	Yes	109	10.90 (1.60)	0.68	14.10 (4.14)	0.18	26.45 (14.60)	0.82
	No	917	10.80 (1.50)		13.98 (3.60)		25.14 (9.80)	
Diabetic foot	Yes	111	11.30 (0.68)	0.005*	14.40 (3.80)	0.005*	27.72 (14.90)	0.034*
	No	915	10.10 (1.70)		13.90 (3.66)		24.51 (13.40)	

*Values are mean (SD), * Significant p value, MPV- mean platelet volume (8-12fl); PDW- platelet distribution width (9-14fl); P-LCR- platelet large cell ratio (11.9-45 %), N= Number*

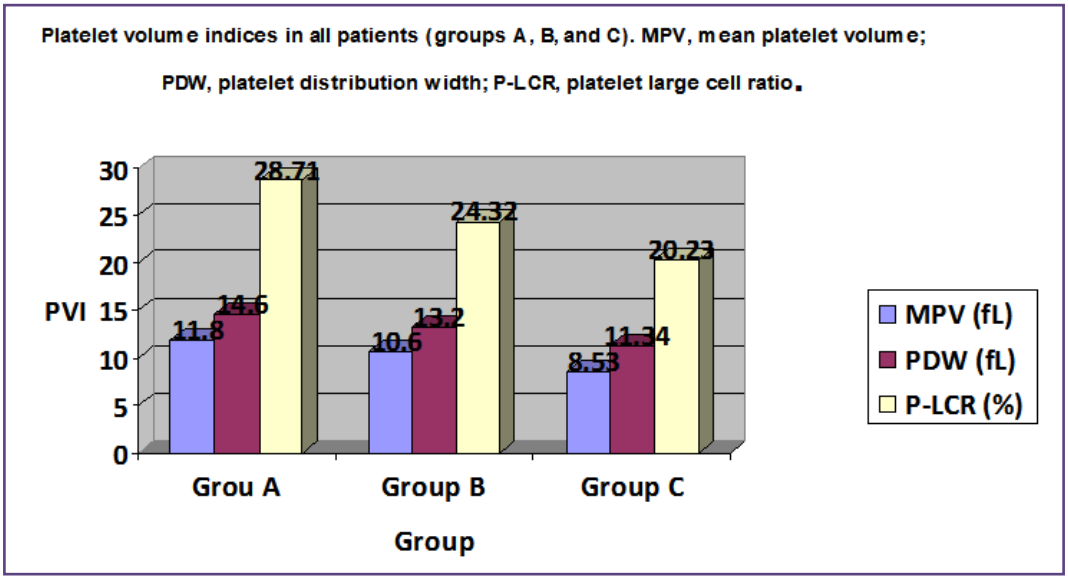

Fig. 1: Platelet volume indices in all patients (groups A, B, and C). MPV, mean platelet volume; PDW, platelet distribution width; P-LCR, platelet large cell ratio.

Discussion

Diabetes is a growing health problem associated with increased risk of micro- and macro-vascular complications. [12] With the easy availability of various blood tests such as platelet volume indices (PVI), efforts are made to identify and prove their utility to act as biomarkers for early detection of diabetic complications.

We found that platelet count was decreased in diabetic patients with complication as compared to control group. However, platelet count did not show any statistical significance on diabetic patients without complications. [13] Insulin resistance and hyperglycemia are said to be the important factors causing increased platelet reactivity in diabetic patients. Platelet hyper reactivity is a well-known contributing factor to the prothrombotic state in diabetics, hence causing increased coagulation, impaired fibrinolysis, and endothelial dysfunction. These hyperactive platelets play a critical role in the pathophysiology of the thrombotic events leading to diabetic complications. [14]

MPV is a parameter used to assess platelet size, and it is a potential biomarker of platelet reactivity. It has been shown that larger platelets are more reactive than smaller ones. [15] PDW can directly measure the variability in platelet size, and its high values suggest increased production of larger reticulated platelets. [16]

Our study suggests that increased MPV, PDW, P-LCR is associated with poor glycometabolic control and it is also reflected in many complications such as retinopathy, nephropathy, neuropathy, CAD and diabetic foot.

Several studies indicated positive correlation of FBS and HbA1c with platelet indices. [17, 18] However, some studies have not shown any relation of FBS and HbA1c with platelet indices. [19,20] It has been proposed that increase in MPV could be because of raised blood sugar leading to osmotic swelling and shorter life span of platelets in diabetic patients. Alternatively, this may suggest that platelet activation is related to glycemic control. [21]

We found that duration of development of complication in diabetes has a significant relation with increased MPV, PDW, P-LCR and Platelet count as agreed by others. [20] Discordant results were also found in other studies. [22] Platelet number and reactivity along with the cardiovascular comorbidities such as hypertension, albuminuria, obesity, cigarette smoking, and dyslipidemia also contributes to the progression of diabetes and its effect on platelet indices. Thus, it shows that there are many other factors which may account for the thrombotic potential of diabetics with time. [23]

Insulin resistance plays a pivotal role in the development of diabetic dyslipidemia by influencing several factors. In insulin resistance and Type 2 diabetes, increased efflux of free fatty acids from adipose tissue and impaired insulin mediated skeletal muscle uptake of free fatty acids increase fatty acid influx into the liver. A cluster of interrelated plasma lipid and lipoprotein abnormalities associated with alterations in very LDL metabolism contribute to the risk for atherosclerosis and CAD in the majority of patients with Type 2 diabetes. Insulin resistance plays a key role in the development of diabetic dyslipidemia. [24] Altered lipid metabolism plays a major pathophysiological role

in diabetes, which can lead to the development of various diabetic complications.

We found statistically significant correlation of MPV with microvascular complications such as diabetic retinopathy and diabetic nephropathy; [19,20] similarly higher values were also seen in the studies done by Dindar *et al*., Ates *et al*. [12,25] MPV was also associated with retinal neovascularization of diabetic retinopathy. Platelets in diabetics are active and have increased aggregation because of dysregulated signaling pathway. This contributes to thrombus formation and microcapillary embolization. The release of constrictive, oxidative, and mitogenic substances such as platelet-derived growth factor and vascular endothelial growth factor accelerates the progression of local vascular lesions such as neovascularization of lens in diabetic retinopathy.

On the other hand, MPV was not significantly different in patients with these complications in studies done by Demirtunc *et al*. [17]

The role of PDW specifically in patients with diabetes and its complication is yet to be explored. Similarly, the P-LCR is not often quoted in the literature, probably because it is a relatively new PVI parameter.

Conclusion

We all are aware of the risk factors for diabetic complications such as duration (3-10 Years), glycemic control, blood pressure, and dyslipidemia. We found increase in MPV, PDW and P-LCR in all these high risk groups. This implies that raised MPV, PDW and P-LCR can be considered as biomarkers for early detection of impending complication. We found that these platelet indices were more statistically significant in microvascular complications as compared to macrovascular complications.

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