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Study of Platelet Indices as a Predictive Parameter for Diabetic Complications in Type II Diabetes Mellitu-A Cross Sectional Study

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ABSTRACT

Introduction: Diabetes Mellitus is a prothrombotic state with enhanced platelet activity. The enhanced platelet activity may result in the development of microvascular and macrovascular complications. Platelet indices i.e. Mean Platelet volume (MPV), Platelet Distribution Width (PDW) are routinely available in alllaboratories and can be used to study as a prognostic marker for the patients.

Aim: To evaluate platelet indices in patients with type 2 diabetes mellitus.

Methods and Material: Cross sectional study was conducted in the Department of Pathology at a tertiary care centre over a period of 18 months. Samples of 120 patients of type 2 diabetes with and without complicationswere investigated. Haematological (platelet count and platelet indices i.e. Mean platelet indices and Platelet distribution width) and biochemical parameters (Fasting blood sugar and HbA1c) were compared between diabetic patients with and without complications. Platelet indices were measured using automated haematology analyser. Statistical evaluation was performed using SPSS version 16.0.

Results: In DM with complication17 (28.33%) out of 60 cases were between the age group of 51-60 years, 14 (23.33%) out 60 belonged to the age group of 61-70 years and 12 (20%) patients were >70 years of age. The mean platelet volume was 12.74±3.076 and 8.65±1.58 for diabetes with and without complications. The mean platelet distribution width was 15.54 and 13.94±2.66 for diabetes with and without complications. The mean HbA1c was 8.33 and 6.75 for diabetes with and without complications. The mean platelet count was 3.12 Lac/mm³ and 2.45 Lac/mm³ for diabetes with and without complications. The mean fasting blood sugar was 219.65 and 109.96 for diabetes with and without complications.

Conclusion: Diabetes is responsible for endothelial dysfunction and platelet hyperactivity. In our study, diabetic patients with uncontrolled glycaemic index and raised fasting blood sugar level had raised platelet indices in comparison to patients without complication where the platelet count, platelet indices level and glycaemic index were within normal limits. These indices can also be used as a prognostic tool.

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Introduction

Diabetes mellitus is group of metabolic disorder sharing the common feature of hyperglycaemia caused by defects in insulin secretion, insulin action or both. It is the leading cause for end stage renal disorder, adult-onset blindness and non-traumatic lower extremity amputation. At present, the prevalence of diabetes in Indian adults is 8.8% [1,2].

The complications associated with Type 2 DM is mainly due to poor glycometabolic control. Glycometabolic control can be monitored by Fasting Blood Glucose (FBG) and Haemoglobin A1c (HbA1c). HbA1c is a useful marker to determine mean

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blood glucose levels over the period of three months. Another cofactor responsible for diabetic complications is altered platelet morphology, increased platelet dysfunction and reactivity which results in the prothrombotic state of DM leading to vascular complications which in turn increases morbidity and mortality in diabetic patients [3,4].

Platelets in DM are hyperactive and releases more granules causing increase in platelet turnover and release of larger platelets which are enzymatically and metabolically active and have a tendency to form clots leading to both macro and microvascular complications [4,5].

Platelet indices can be measured by automated haematology analysers (Nihon Kohden) as a routine haematological procedure

[6]. The Mean Platelet Volume (MPV) in our lab is between: 7.4-10.4 fL and Platelet Distribution Width (PDW) is 9-14 fL. MPV is indicator of the average size and activity of platelets. It is calculated by dividing the Platelet Crit (PCT) by platelet count. Variability in the platelet size can be measured by PDW. It is measurement by calculating the width of the platelet histogram at the 20% height level. PDW is elevated earliest than other indices [7,8]. In our study the effects of long-standing diabetes were studied for its complications and its emphasis on platelet function.

Objectives

- To study platelet indices (platelet count, MPV, PDW) as a predictive biomarker for type II Diabetes mellitus.
- To study clinical parameters in DM patients with and without complication.
- To Evaluate Diabetic related complication.
- To study the levels of HbA1c and FBS levels in complicated and uncomplicated cases of DM

Materials and Methods

This was a cross sectional study which was carried out in the Department of Pathology in a tertiary care centre. Data was collected over a period of 24 months (July 2020 to May 2022). Blood was collected into K_2 EDTA tubes and platelet parameters were measured within 1 hour after venepuncture. The Mean Platelet Volume (7.4-10.4 fL) and Platelet Distribution Width (9-14 fL) were studied in diabetic patients with and without complications and comparison between platelet value and HbA1c was carried. The microvascular and macrovascular complications were noted. All the data was compiled in MS-excel and analysed further. A sample size of 120 was collected and formula used was

$$N = (SD1^{2} + SD2^{2})(Z_{1}-\alpha/2 + Z_{1}-\beta)$$
$$M_{1} - M_{2}$$

S.D – Standard Deviation

 $(Z1-\alpha/2 + Z1-\beta)$ – Constant i.e 7.84

 M_1 – Platelet indices level in diabetic patient with complication M_2 – Platelet indices level in diabetic patient without complication

Analysis of data was done using SPSS software. ANOVA test, Chi – square test was applied and P value was calculated. This work was approved by Institutional Ethic Committee (Protocol Reference number KIMS/ 030/2020-21).

Inclusion Criteria: Patients with Type II DM with and without complications.

Exclusion Criteria: Patients with other systemic diseases along with DM (Hypertension, SLE, RA, patient on corticosteroids, Cushing syndrome, any diagnosed malignancy patients, patients on antiplatelet drugs such as aspirin etc).

Normal Range: MPV: 7.4-10.4 fL, Platelet Distribution Width (PDW): 9-14 fL,FBS: <100 mg/dL, HbA1c: <6.5%

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0. Qualitative data were described as number and percentage. Quantitative data were described as range (maximum and minimum), mean and standard deviation. Comparison between different group regarding categorical variable was performed using the Student's t-test and Pearson's correlation test (r value

as the coefficient) were used. p-value (probability)<0.05 was considered significant.

Results

Age Distribution of DM with and without Complications

Total number of cases of DM with complications, 17 (28.33%) out of 60 patients were between the age group of 51-60 years, 14 (23.33%) out 60 belonged to the age group of 61-70 years and 12(20%) out of 60 were >70 years of age. In patients of DM without complications, 21 (35%) out of 60 patients were between the age group of 51-60 years, 5 (8.33%) out of 60 belonged to the age group of 61-70 years and 8 (13.3%) out of 60 were >70 years of age.

Table 1: Age	Wise	Distribution	of	DM	with	and	without
Complication							

Age (in Years)	DM PI Complicated (n=60)	DM PI Without Complicated (n=60)
21-30	3(5%)	2(3.33%)
31-40	4(6.67%)	4(6.67%)
41-50	10(16.67%)	20(33.33%)
51-60	17(28.33%)	21(35%)
61-70	14(23.33%)	5(8.33%)
>70	12(20%)	8(13.33%)

Table 2: Comparison of Age Wise Distribution in Other andPresent Study

Study Series	Males in %	Females in %	Mean Age (Years)
In Our Study	63.33	36.67	57.49 ± 14.48
Bhattacharjee P et al. [9]	51	49	57.3 ± 14.18
Yenigun et al. [10]	31.3	68.7	59.35 ± 9.04
Kodiatte et al. [5]	65	35	55 ± 11.2

Comparing Platelet Indices with HbA1c Levels in Patients of DM with Complications

The MPV was 11.95 ± 2.55 for HbA1c <7.5 (n=21), 12.20 ± 2.88 for 7.5 to 10 HbA1c (n=29) and 15.91 ± 3.35 for >10 HbA1c (n=10). The mean PDW was 15.10 ± 1.56 for <7.5 HbA1c, 16.08 ± 2.11 for 7.5 to 10 HbA1c and 17.00 ± 1.99 for >10 HbA1c. The mean platelet count was 2.93 Lac/mm³ ±1.11 Lac/mm for HbA1c <7.5, 3.54 Lac/mm³ ±1.12 for 7.5 to 10 HbA1c and 3.67 Lac/mm³ ±1.14 Lac/mm³ for >10 HbA1c.

 Table 3: Comparison of Platelet Indices with HbA1c Levels

 in DM with Complications

Platelet Indices DM with Complications							
HbA1C	MPV		PDW		Platelet	Platelet Count	
	Mean	SD	Mean	SD	Mean	SD	
<7.5 (n=21)	11.95	2.55	15.10	1.56	293762	111021	
7.5 to 10 (n=29)	12.20	2.88	16.08	2.11	354095	112362	
> 10 (n=10)	15.91	3.35	17.00	1.99	367510	114903	
ANOVA <i>F-value</i>	7.51	1	4.0	1	3.2	257	
p-value	0.001	3*	0.0235*		0.04	58*	

Graph 1: Line Chart Showing Platelet Indices and platelet Count as per HbA1c Levels in DM with Complications



Comparing Platelet Indices in DM without Complications

The MPV for DM without complication was 8.63 ± 1.51 for HbA1c <7.5 and 8.83 ± 2.05 for 7.5 to 10 HbA1c. The mean PDW for DM without complications was 13.88 ± 2.77 for HbA1c <7.5 and 14.23 ± 2.09 for HbA1c 7.5 to 10. The mean platelet count for DM without complication was 2.48 Lac/mm $\pm83,398$ for HbA1c <7.5 and 2.25 Lac/mm $\pm78,757$ for HbA1c 7.5 to 10.

Table 4: Comparison of Platelet Indices with HbA1c in DM Patients without Complications

Platelet Indices in DM without Complications							
HbA1C	MP	PV P		PDW Platele		Count	
	Mean	SD	Mean	SD	Mean	SD	
<7.5 (n=51)	8.63	1.51	13.88	2.77	248843.1373	83398.4	
7.5 to 10 (n=9)	8.83	2.05	14.23	2.09	225555.5556	78757.7	
unpaired t-value	0.35		0.36		7.428		
p-value	0.7	3	0.7	71	<0.0001*		

Table 5: Comparison of Mean Platelet Volume in Other andPresent Study

Study Series	Mean Platelet Vo	P Value	
	Diabetics with Complications	Diabetics without Complications/ Control	
Our Study	12.74±3.076	8.65±1.58	<0.0001*
Bhattacharjee P et al. [10]	12.65 ± 1.89	11.16 ±1.18	0.03
Hekimsoy et al. [11]	10.62±1.71	9.15±0.86	0.000

Table 6: Comparison of Platelet Distribution Width in Other and Present Study

Study Series	Platelet Distribut	P Value	
	Diabetes with Complications	Diabetes Without Complications/ Control	
Our Study	15.54	13.94	0.0040
Demirtas et al. [12]	16.4	15.4	<0.001
Jabeen et al. [13]	15.02	14.12	0.003
Dalamaga et al. [14]	16.4	13.0	<0.001

Comparison of HbA1c and FBS in Diabetic Patients with and without Complications

The mean HbA1c was 8.33 ± 1.45 for Diabetes with complication and 6.75 ± 0.61 for diabetes without complications. The mean FBS was 219.65±101.32 for Diabetes with complication and 109.96±26.17 for diabetes without complications.

Graph 2: Line Chart Showing Mean Comparison of MPV, PDW, HbA1c, Platelet Count and FBS in DM Patients with and without Complications



Comparison of Platelet Indices and Platelet Count in Patients of Diabetes Mellitus with Complications

The mean platelet count was 2.97 ± 1.022 Lac/mm³ in stroke, 2.0 Lac/mm³\pm64500 in MI, 4.09 ± 1.15 Lac/mm³ in AKI, 2.71 Lac/mm³±69429 in CKI, 3.70 Lac/mm³±1.13 Lac/mm³ in gangrene toe, 3.52 Lac/mm³ ± 1.07 Lac/mm³ in diabetic retinopathy and 3.19 Lac/mm³ ±58174 mm³ in Peripheral vasculopathy. The mean PDW was 13.48 ± 3.28 fL in stroke, 12.05 ± 1.27 fL in MI, 13.78 ± 1.38 in AKI, 14.05 ± 2.29 in CKI, 14.8 ± 3.15 fL in gangrenous toe, 14.1 ± 3.31 fL in diabetic retinopathy and 11.86 ± 1.067 fL in Peripheral vasculopathy. The mean platelet volume was 13.98 ± 1.37 in stroke, 12.82 ± 0.12 in MI, 12.07 ± 0.98 in AKI, 11.56 ± 0.94 in CKI, 12.48 ± 1.43 in gangrene toe, 13.02 ± 1.29 in diabetic retinopathy and 10.36 ± 0.72 in Peripheral vasculopathy.

 Table 7: Comparison of Platelet Indices, Platelet Count Fasting Blood Sugar and HbA1c in Patients of Diabetes Mellitus with Complications

Complications	Platelet Count	PDW	HbA1c	MPV	FBS
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Stroke (23.33%)	297937.5±102230	13.48±3.28	14.55±2.98	13.98±1.37	213.06±131.4
MI (6.66%)	200250±64500	12.05±1.27	16.27±1.37	12.82±0.12	182.5±23.62
AKI (15%)	409142.8±115132	13.78±1.38	16.17±0.95	12.07±0.98	291.42±101.23
CKI (13.33 %)	271777.7±69429	14.05±2.29	17.58±0.59	11.56±0.94	215.5±73.67
Gangrenous toe (13.33%)	370416.6±113023	14.8±3.15	18.21±0.93	12.48±1.43	240±88.93
Diabetic Retinopathy (10%)	352313.1±107119	14.1±3.31	15.62±0.84	13.02±1.29	182.9±78.93

Peripheral Vasculopathy (10%)	31966.6±58174	11.86±1.067	15.9±1.17	10.36±0.72	130±32.24
ANOVA F-value	3.716	2.295	6.206	1.947	2.003
p-value	0.0064	0.0599	0.0002	0.004	0.0095

Discussion

Diabetes Mellitus is a major health problem worldwide, characterised by increase in the prothrombotic activity resulting in accelerated atherosclerosis, inflammation and enhanced platelet activity, significantly involving multi-organs such as heart, nerves, eyes, CNS, kidneys, gastrointestinal tract and blood vessels, leading to long-term complications owing to increased mortality and morbidity [15-18].

The platelet in DM is immature, larger and have increased activity. Hyperglycaemia directly causes increase in platelet reactivity and promotes glycation of platelet proteins. Both insulin resistance and insulin deficiency increase the platelet reactivity. Insulin inhibits activation of platelets. Therefore, relative or absolute deficiency of insulin would increase platelet reactivity. In patients with diabetes, there is platelet hyperaggregability and platelet activation causing the circulating platelets to release more granules which reduce the survival of platelets and hence releasing larger platelets from bone marrow due to the activation of megakaryocytes. These larger and younger platelets have large volume and they are functionally active because they have increased surface marker on their surface. Enhanced platelet aggregation and activation results in the development of various microvascular and macrovascular complications [13].

These large platelets can be detected by haematology analysers and giving us the reading for MPV and PDW. Thereby monitoring of platelet function in diabetic patient via platelet indices can help in curbing diabetic related morbidity and mortality.

Age Distribution

In our study the mean age of the study population was 57.49 ± 14.48 years. DM with complications were seen in higher age groups i.e. 17 (28.33%) out of 60 patients belonged to the age group of 51-60 years, 14 (23.33%) out 60 belonged to the age group of 61-70 years and 12(20%) out of 60 were>70 years of age.

In the study by Spandana. T et al. Shilpi K, Potekar RM and Bhattacharjee P et al, mean age was 55,53 and 57 years in patients with DM with complications which was concordant with our study as shown in Table 2 [15,19].

Platelet Indices

In our study the mean MPV and PDW for DM without complications was 8.63 ± 1.58 and 13.94 ± 2.66 and is concordant with the study by Shilpi K, Potekar RM [14]. The mean MPV and PDW for DM with complications was 12.74 ± 3.076 and 15.54 ± 3.31 and is concordant with the study by Hekimsoy et al., Bhattacharjee P et al., Shilpi K, Potekar RM and Sonali Jindal et al. as shown in the table 4 and 5 [11,15,19,20].

Platelet Count

In our study the mean platelet count for DM with complication was $3.12 \text{ Lac/mm}^3 \pm 1.07 \text{ Lac/mm}^3$ and is concordant with study by Sonali Jindal et al. which was $229.33 \pm 70,000 \text{ Lac/mm}^3$ [21].

Gylcosylated Hemoglobin

In our study the mean HbA1c for DM with complications was 8.33 ± 1.45 . The mean HbA1c in patients of DM without complication was 6.75 ± 0.61 which is concordant with study by Spandana T et al. and Shilpi K, Potekar RM having HbA1c levels 7.28 ± 0.88 and 7.3 ± 1.1 [19].

Fasting Blood Sugar

We have studied the correlation of FBS with its role on vasculopathy. The mean FBS was 219.65 ± 101.32 and 109.96 ± 26.17 for DM with and without complications respectively and is concordant with the study by Shilpi K, Potekar [19].

Platelet Count in DM with Complications

The mean platelet count in this study was 2.97 ± 1.022 Lac/mm³ in stroke, 2.0 Lac/mm³± 64500 in Myocardial infarction, 4.09 ± 1.15 Lac/mm³ in Acute kidney injury, 2.77 Lac/mm³± 69429 inChronic kidney injury, 3.72 Lac/mm³±1.13 in gangrene toe and 3.19 Lac/mm³± 58174 inPeripheral vasculopathy and is concordant with the study by Shilpi K, PotekarRM and Walinjkar [19,22]. In our study platelet count was increased and we studied various systemic complications in diabetic patients with the values of platelets.

Platelet Indices in DM With Complications

The mean PDW was 13.48 ± 3.28 in Stroke, 12.05 ± 1.27 in Myocardia I infarction, 13.78 ± 1.38 in Acute kidney injury, 14.05 ± 2.29 in Chronic kidney injury 14.8 ± 3.15 in gangrenous toe, 14.1 ± 3.31 in Diabetic retinopathy and 11.86 ± 1.067 in Peripheral vasculopathy and is concordant with the study by Shilpi K, Potekar RM and Walinjkar. The mean platelet volume was 13.98 ± 1.37 in Stroke, 12.82 ± 0.12 in MI, 12.07 ± 0.98 in Acute kidney Injury, 11.56 ± 0.94 in Chronic kidney Injury, 12.48 ± 1.43 in gangrene toe, 13.02 ± 1.29 in diabetic retinopathy and 10.36 ± 0.72 in Peripheral vasculopathy and was concordant with the study by Shilpi K, Potekar RM and Walinjkar et al. [19, 22].

Limitation

The limitations of this study were that no follow up with of the few cases and making it unable to compare them with present findings. However, these factors constitute minimal number of cases in this study. Patients with qualitative disorders and reactive causes for raised platelets were not assessed that constitute a minor role.

Conclusion

Diabetes is responsible for endothelial dysfunction and platelet hyperactivity, which results in microvascular and macrovascular complications. The platelets in DM are larger, active and have higher thrombogenic potential and results in raised platelet indices. In our study, variations in levels of platelet count and platelet indices between diabetic patients with and without complications were observed. Diabetic patients with uncontrolled glycaemic index and raised fasting blood sugar level had raised platelet indices leading to complications in comparison to patients without complication where the platelet count, platelet indices level and glycaemic index were within normal limits.

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