STUDY OF MEAN PLATELET VOLUME IN INDIVIDUALS WITH TYPE II DIABETES MELLITUS AND ITS CORRELATION WITH HbA$_1$c

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ABSTRACT

To study MPV in patients of Type 2 Diabetes Mellitus. To correlate MPV with diabetic control as indicated by HbA1c. Platelet count of the patients having diabetes under control and uncontrolled diabetes was found to be significantly high compared to non diabetic patients. MPV levels increased consistently and linearly with increase in FBS, PPBs and HbA1c levels and correlate at 5% significance level.

Keywords
Mean platelet volume , hba1c , diabetes mellitus.

Introduction

Platelets are essential for haemostasis, and knowledge of their function is basic to understanding the pathophysiology of vascular disease in diabetes(1). MPV is a physiological variable of haemostatic importance(2). Large platelets are more reactive, have a greater content of granules, release more serotonin and β–thromboglobulin than do small platelets, produce more thromboxane A2, aggregate more easily and can therefore exert their haemostatic, vasomotor and pro-inflammatory functions with greater efficacy(3). Also, increased MPV has been associated with greater in vitro aggregation in response to ADP and collagen(4). Increase in platelet volume has been reported in acute myocardial infarction, acute cerebral ischemia, and transient ischemic attack, chronic vascular disease (5,6). In addition, there is evidence that an elevated MPV is associated with a poor outcome among survivors of myocardial infarction and stroke (7,8). Platelets play a crucial role in the pathogenesis of atherosclerotic complications, contributing to thrombus formation or apposition after plaque rupture. Moreover, increased platelet size has been reported in patients with vascular r isk factors such as hypercholesterolemia and smoking (9).

There are basically three parameters for understanding platelet function and morphology that is Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Large Cell Ratio (P- LCR).MPV and PDW are easily measured platelet indices, which increase during platelet activation. In order to obtain a larger surface, platelets change in shape during activation. Their shape changes from discoid to spherical. Pseudopodia are formed as well (10). MPV range from 8 -12 fL and PDW range from 9-14 fL. P-LCR (Platelet large cell ratio) indicates percentage of large platelets with volume › 12 fL, standard range is 15-35%. Type 2 Diabetes Mellitus is characterised mainly by tissue insulin resistance and impaired insulin secretion. Platelet hyperactivity and increased baseline activation in patients with diabetes is multifactorial and associated with biochemical factors such as hyperglycaemia and hyperlipidaemia , insulin resistance and inflammatory and oxidant state (11). Increased platelet activity due to abnormal insulin action is emphasised in the development of vascular complications of this disease. Higher value of MPV has been shown in diabetic patients in parallel to the micro vascular complications such as retinopathy and micro albuminuria (12,13). Insulin is natural antagonist of plate let hyperactivity. It sensitizes the platelet to PGI2 and enhances generation of PGI2 and NO. Thus the defect in nsulin action in diabetes creates a milieu of

disordered platelet conducive to macro vascular and micro vascular events (1). Increase in MPV has been found in various studies done by Papanas N et al (12), Hekimsoy Z et al (13) and Zuberi et al (14). All studies have found increase in MPV in diabetics as compared with non-diabetics. Ate O et al also found similar results in patients of diabetic retinopathy(15). As there on only few studies based on MPV AND HbA1c in this part of our country, we correlated diabetes control (as defined by HbA1c) with MPV, which has been done only in few studies.

MATERIALS AND METHODS

Study Set Up:

The study was conducted in the Department of General Medicine SBMCH, CHENNAI.
Study Design:

CASE CONTROL study.Done from December 2015 to September 2016.
Study Subjects:

60 patients of Type 2 Diabetes Mellitus attending Medicine Opd and those admitted in ward with at least 6 months of duration of disease were taken as subjects.
Study Controls:

40 age and sex match healthy controls were taken.

Consent and Ethical clearance:

Written and informed consent was taken from all the subjects participating in study. Ethical clearance was taken from ethical committee before conducting the study.
Inclusion Criteria:

- Either gender

- Age > 30 yrs

- Patients of Type 2 Diabetes Mellitus of at least 6 months duration with diagnosis made on basis of ADA 2013.

*In testingthe absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

- Males with Hb> 11 gm% and Females with Hb> 10 gm%.

Exclusion Criteria:

Subjects with the following conditions were excluded

- Patients on antiplatelet drugs

- Idiopathic Thrombocytopenic Purpura

- Septicaemia

- Pregnancy

- Other underlying life threatening illnesses

- End stage renal disease

- Cirrhosis and Fulminant hepatic failure.

- Male patients with Hb less than 11gm% and female patients with Hb less than 10 gm% because nutritional anaemia can be a cause of reactive thrombocytosis and hence increased MPV.

METHODOLOGY

Sample Collection and Analysis

Blood (2 ml) for platelet indices was collected in dipotassium EDTA tubes on admission by a clean puncture, avoiding bubbles and froth. The sample will be run within two hours of venipuncture using the Sysmex XT-2000 automated cell counter. During the time between venipuncture and processing, the samples were stored at room temperature. The different blood tubes were used for all patients. Samples for plasma glucose estimation and HbA1c were taken in sodium fluoride and EDTA vial respectively. Estimation of FBS and PPBS was carried out by glucose oxidase method in auto analyser (Hitachi 902) and that of HbA1c by ion exchange chromatography method.

| Group	Intervention
Group A	Diabetes under control
Group B	Uncontrolled Diabetes
Control Group	Non Diabetic

DATA ANALYSIS:

Descriptive statistics was done for all data and suitable statistical tests of comparison were done. Continuous variables were analysed with the unpaired and paired t test and categorical variables wereanalysed with the Chi-Square Test and Fisher Exact Test. Correlation analysis done using pearsons r correlation and ANOVA. Statistical significance was taken as $P < 0.05$. The data was

analysed using EpiInfo software (7.1.0.6 version; Center for disease control, USA) and Microsoft Excel 2010.

RESULTS

Table 1 : HbA1c Distribution – Groups

Groups	Group A	Group B	Control
Description	Diabetics with HBA1c $\leq$ 7%	Diabetics with HBA1c $>$ 7%	Non Diabetics
Number	24	36	40
Mean	6.48	10.03	5.82
SD	0.34	2.96	0.74
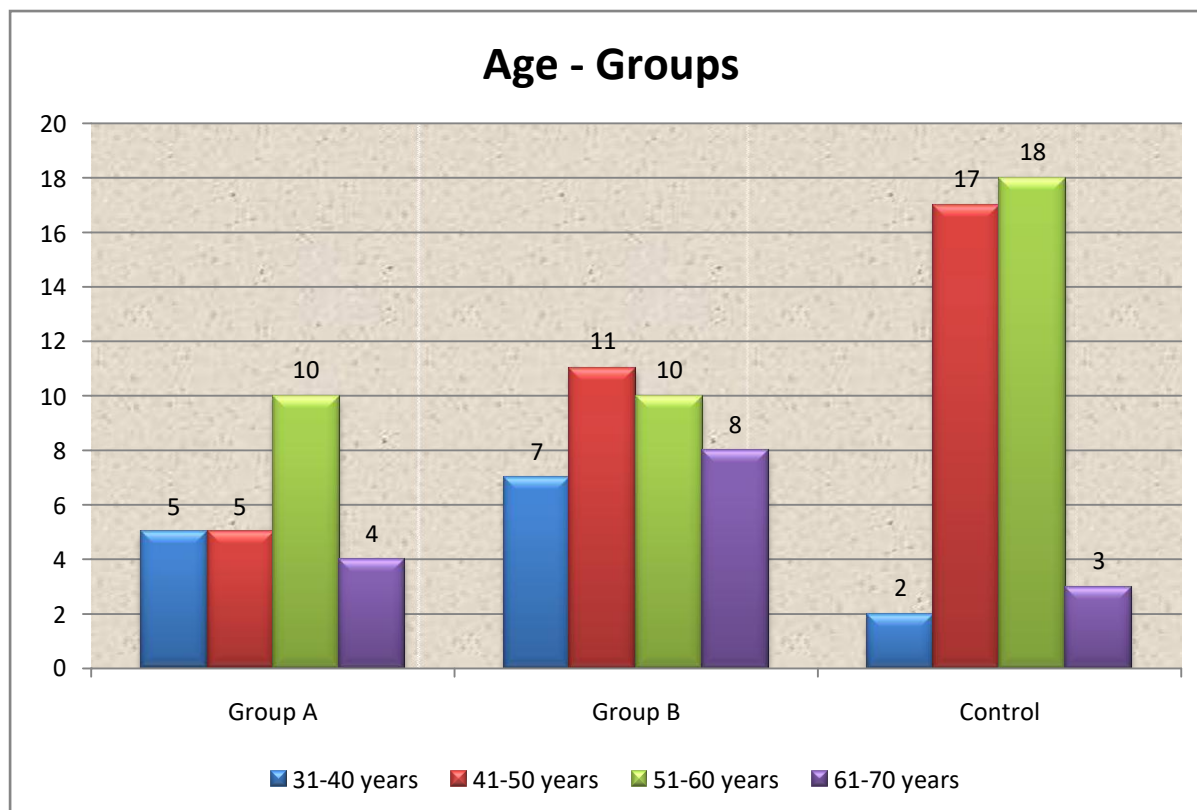
FIG 1: Shows the AGE-groups

Table 2 : Age Distribution

Age Distribution	Group A	Group B	Control
Mean	50.92	50.50	50.85
SD	9.91	11.18	6.06
P Value Unpaired t Test	Group A Vs Group B		0.8830
	Group A Vs Control		0.9734
	Group B Vs Control		0.8639

Majority of the Group A patients belonged to the 51 - 60 years age class interval (n=10, 41.67%) with a mean age of 50.92 years. In Group B patients, majority belonged to the 41-50 years age class interval (n=11, 30.56%) with a mean age of 50.50 years. In the Control Group patients, majority belonged to the 51 -60 years age class interval (n=18, 45.00%) with a mean age of 50.85 years. The association between the study groups and age distribution is considered to be not statistically significant since $p > 0.05$ as per unpaired t test.

FIG 2: Shows the gender status
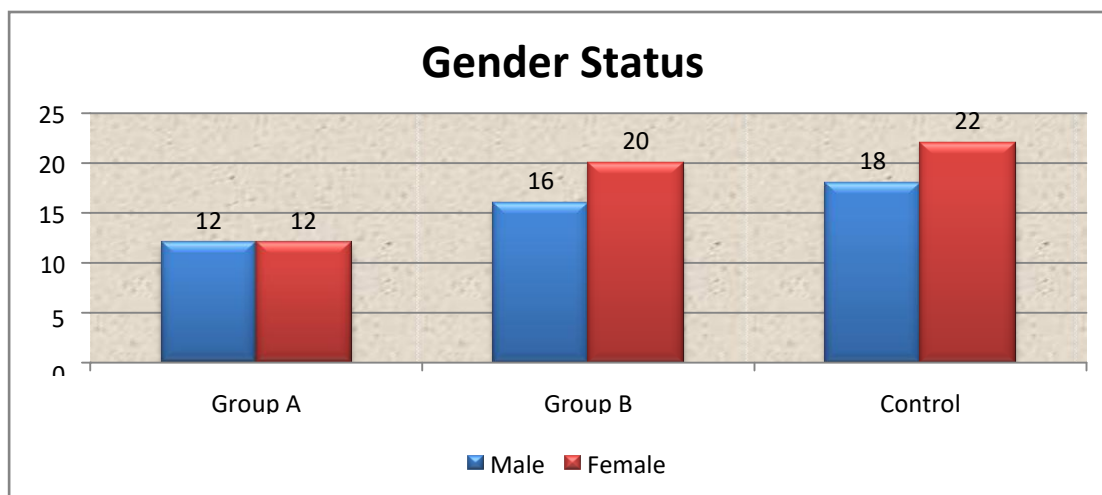
The Group A patients belonged equally to male and female gender (n=12, 50.00%). In Group B patients, majority belonged to female gender (n=20, 55.56%) followed by male gender (n=16, 44.44%). In the Control Group patients, majority similarly belonged to female gender (n=22, 55.00%) followed by male gender (n=18, 45.00%).The association between the study groups and gender status is considered to be not statistically significant since $p > 0.05$ as per chi squared test.

Table 3 : FBS - Groups

FBS - Groups	Group A	Group B	Control	Group A (%)	Group B (%)	Control (%)
≤ 100 mg/dL	8	1	35	33.33	2.78	87.50
101 -150 mg/dL	13	17	5	54.17	47.22	12.50

151-200 mg/dL	3	15	0	12.50	41.67	0.00
> 200 mg/dL	0	3	0	0.00	8.33	0.00
Total	24	36	40	100	100	100

Majority of the group A patients belonged to 101 -150 mg/dL FBS level class interval (n=13, 54.17%) with a mean FBS level of 118.38 mg/dl. In the group B patients, majority belonged to 101 -150 mg/dL FBS level class interval (n=17, 47.22%) with a mean FBS level of 154.75 mg/dl. In the control group patients, majority belonged to ≤ 100 mg/dL FBS level class interval (n=35, 87.50%) with a mean FBS level of 89.48 mg/dl. By conventional criteria the association between the study groups (group A Vs group B, group A Vs control group and group B Vs control group) and FBS levels is considered to be statistically significant since $p < 0.05$ as per unpaired t test.

FIG 3: Shows the PPBS groups
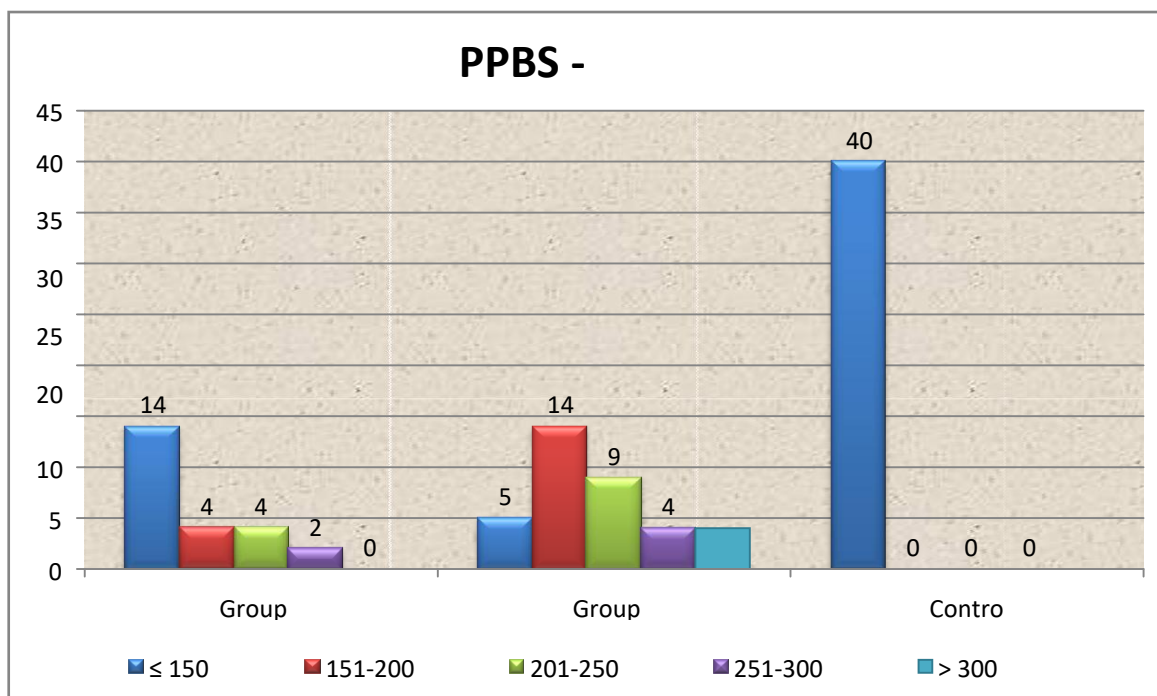
Majority of the group A patients belonged to ≤ 150 mg/dl PPBS level class interval (n=14, 58.33%) with a mean PPBS level of 165.71 mg/dl. In the group B patients, majority belonged to 151 -200 mg/dl PPBS level class interval (n=14, 38.89%) with a mean PPBS level of 213.39 mg/dl. In the control group patients, majority belonged to ≤ 150 mg/dl PPBS level class interval (n=40, 100.00%) with a mean PPBS level of 109.00 mg/dl. By conventional criteria the association between the study groups (group AVs group B, group A Vs control group and group

B Vs control group) and PPBS levels is considered to be statistically significant since $p < 0.05$ as per unpaired t test.

Table 4 : shows the PPBS Distribution

PPBS Distribution	Group A	Group B	Control
Mean	165.71	213.39	109.00
SD	49.24	59.42	10.79
P Value Unpaired t Test	Group A Vs Group B		0.0019
	Group A Vs Control		<0.0001
	Group B Vs Control		<0.0001

Table 5 : shows the Platelet Count – Groups

Platelet Count - Groups	Group A	Group B	Control	Group A (%)	Group B (%)	Control (%)
≤ 250 (X 10 /L)	8	4	27	33.33	11.11	67.50
251-300 (X 10 /L)	12	21	13	50.00	58.33	32.50
301-350 (X 10 /L)	4	11	0	16.67	30.56	0.00
Total	24	36	40	100	100	100

Majority of the group A patients belonged to 251 -300 (X 10 /L) platelet count class interval (n=12, 50.00%) with a mean platelet count of 267.20 (X 10 /L). In the group B patients, majority belonged to 251 -300 (X 10 /L) platelet count class interval (n=21, 58.33%) with a mean platelet count of 284.00 (X 10 /L). In the control group patients, majority belonged to ≤ 250 (X 10 /L)platelet count class interval (n=27, 67.50%) with a mean platelet count of 243.03 (X 10 /L). By conventional criteria the association between the study groups (group A Vs group B, group A Vs control group and group B Vs control group) and platelet count is considered to be statistically significant since $p < 0.05$ as per unpaired t test.

FIG 4: Shows the MPV platelet volume- Groups
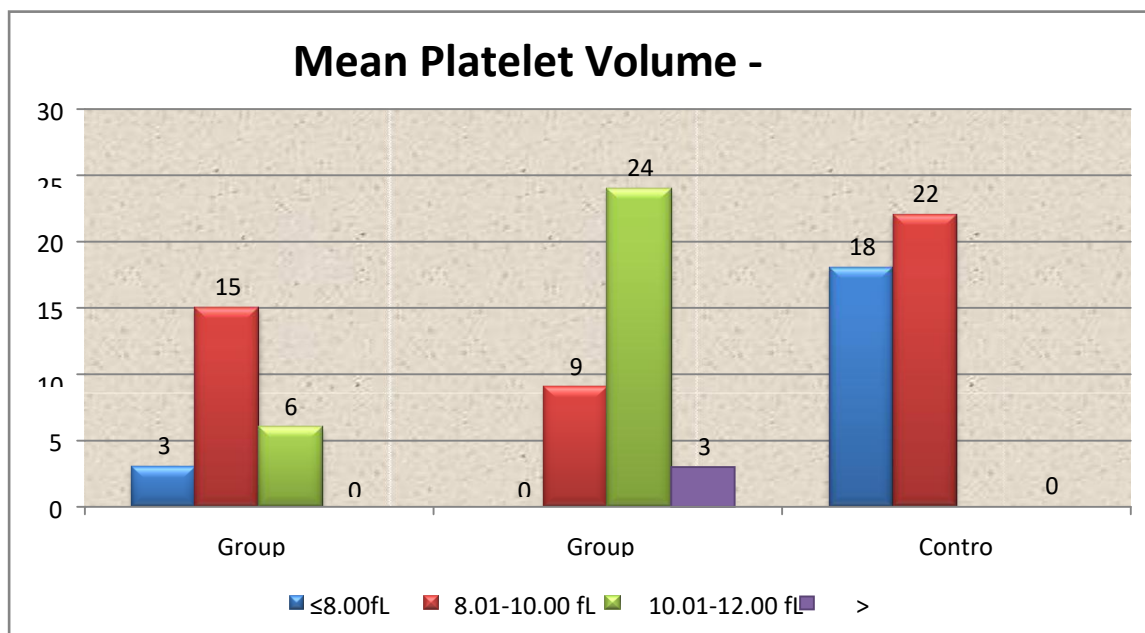
Mean Platelet Volume - Groups	Group A	Group B	Control	Group A (%)	Group B (%)	Control (%)
≤ 8.00 fL	3	0	18	12.50	0.00	45.00
8.01-10.00 fL	15	9	22	62.50	25.00	55.00
10.01-12.00 fL	6	24	0	25.00	66.67	0.00
> 12 fL	0	3	0	0.00	8.33	0.00
Total	24	36	40	100	100	100

Table 6 : shows the Mean Platelet Volume – Groups

Table 7: Mean Platelet Volume Distribution

Mean Platelet Volume Distribution	Group A	Group B	Control
Mean	9.55	10.65	7.98
SD	0.99	0.88	0.41
P Value Unpaired t Test	Group A Vs Group B		<0.0001
	Group A Vs Control		<0.0001
	Group B Vs Control		<0.0001

Majority of the group A patients belonged to 8.01 - 10.00 fL MPV class interval (n=15, 62.50%) with a mean MPV of 9.55 fL. In the group B patients, majority belonged 10.01-12.00 fL MPV class interval (n=24, 66.67%) with a mean MPV of 10.65 fL. In the control group patients, majority belonged to 8.01 -10.00 fL MPV class interval (n=22, 55.00%) with a mean MPV of 7.98 fL. By conventional criteria the association between the study groups (group A Vs group B, group A Vs control group and group B Vs control group) and MPV values is considered to be statistically significant since $p < 0.05$ as per unpaired t test.Correlation of MPV FBS Vs MPV. In patients belonging to our study group, when FBS levels was cross matched against MPV levels, there was a statistically significant as the p value is < 0.0001 with a positive correlation as per pearson's coefficient of 0.5617.

Table 8 : PPBS Vs MPV

Pearson's " r" Correlation	Multiple R	R Square	P Value
PPBS Vs MPV	0.522518	0.273025	<0.0001

In patients belonging to our study group, when PPBS levels was cross matched against MPV levels, there was a statistically significant as the p value is < 0.0001 with a positive correlation as per pearson's coefficient of 0.5225

Table 9 : HBA1c Vs MPV

Pearson's " r" Correlation	Multiple R	R Square	P Value
HBA1c Vs MPV	0.64108	0.494552	<0.0001

In patients belonging to our study group, when HBA1c levels was cross matched against MPV levels, there was a statistically significant as the p value is < 0.0001 with a positive correlation as per pearson's coefficient of 0.6410 .

DISCUSSION

In our study when the FBS levels was matched against the study groups it was observed that, FBS was significantly high in group B compared to group A by a mean difference of 36.38 mg/dl (24% higher), also significantly high in group A compared to control g roup by a mean difference of 28.90 mg/dl (24% higher) and significantly high in group B compared to control group by a mean difference of 65.28 mg/dl (42% higher). The differences were significant with a p-value of<0.0001 as per unpaired t-test. (16-18)In our study the PPBS levels was matched against the study groups it was observed that, PPBS was significantly high in group B compared to group A by a mean difference of 47.68 mg/dl (22% higher), also significantly high in group A compared to control group by a mean difference of 56.71 mg/dl (34% higher) and significantly high in group B compared to control group by a mean difference of 104.39 mg/dl (49% higher). The differences were significant with a p-value of 0.0019,<0.0001and <0.0001 respectively as per unpaired t-test.(19)

In our study when the platelet count was matched against the study groups it was observed that, platelet count was significantly high in group B compared to group A by a mean difference of 16.80 (X 10 /L) (6% higher), also significantly high in group A compared to control group by a mean difference of 24.18 (X 10 /L) (9% higher) and significantly high in group B compared to control group by a mean difference of 40.98 (X 10 /L) (14% higher). The differences were significant with a p-value of 0.0185, 0.0001 and <0.0001as per unpaired t -test.

In our study when the mean platelet volume was matched against the study groups it was observed that, platelet count was significantly high in group B compared to group A by a mean difference of 16.80 (X 10 /L) (6% higher), also significantly high in group A compared to control group by a mean difference of 24.18 (X 10 /L) (9% higher) and significantly high in group B compared to control group by a mean difference of 40.98 (X 10 /L) (14% higher). The differences were significant with a p-value of <0.0001as per unpaired t -test.

When correlation tests were applied between MPV and glycemic parameters it was observed that the increase in levels of MPV correlates positively with the increase in FBS, PPBS and HBA1c levels. The linear increase in MPV level measurement in relation to increased FBS levels is true 56% of times and this variation is truly accounted for 32% of times, also the linear increase in MPV level measurement in relation to increased PPBS levels is true 52% of times and this variation is truly accounted for 27% of times and linear increase in MPV level measurement in relation to increased HBA1c levels is true 64% of times and this variation is truly accounted for 49% of times.(20)

CONCLUSION

The FBS levels of the patients having diabetes under control and uncontrolled diabetes was found to be significantly high compared to non diabetic patients. it is also noted that FBS levels of the patients having uncontrolled diabetes was found to be significantly high compared to patients with diabetes under control. In other words FBS levels were 1.31 times more in uncontrolled diabetes patients compared to patients with diabetes under control, 1 .32 times more in patients with diabetes patients compared to non diabetics and 1.73 times more in uncontrolled diabetes patients compared to non diabetics. The PPBS levels of the patients having diabetes under control and uncontrolled diabetes was found to be significantly high compared to non diabetic patients. it is also noted that FBS levels of the patients having uncontrolled diabetes was found to be significantly high compared to patients with diabetes under control.

The platelet count of the patients having diabetes under control and uncontrolled diabetes was found to be significantly high compared to non diabetic patients. it is also noted that platelet count of the patients having uncontrolled diabetes was found to be significantly high compared to patients with diabetes under control. In other words platelet count were 1.06 times more in uncontrolled diabetes patients compared to patients with diabetes under control, 1.10 times more in patients with diabetes patients compared to non diabetics and 1.17 times more in uncontrolled diabetes patients compared to non-diabetics patients. It is also noted that platelet count of the patients having uncontrolled diabetes was found to be significantly high compared to patients with diabetes under control. In other words platelet count were 1.12 times more in uncontrolled diabetes patients compared to patients with diabetes under control, 1.58 times more in patients with diabetes patients compared to non diabetics and 1.68 times more in uncontrolled diabetes patients compared to non diabetics. MPV levels increased consistently and linearly with increase in FBS, PPBs and HbA1c levels and correlate at 5% significance level.

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

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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