

Original Research Article

A study to find correlation of platelet indices with HbA1c in diabetic patients with absence/presence of vascular complications

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ABSTRACT

Background: Diabetes Mellitus is a global pandemic disease. The increased platelet activity is emphasized to play a role in the development of vascular complications of the metabolic disorder. Mean platelet volume (MPV) is an indicator of average size and activity of platelets. This study was conducted to find correlation of platelet indices with HbA1c in diabetic patients with absence/presence of vascular complications.

Methods: Total of 100 subjects was enrolled in the study. Sample for glucose estimation and platelet indices were collected and estimation were carried out by the auto-analyzers. The statistical evaluation is done using SPSS version 22. Student t- test was used for doing comparison between two variables namely HbA1c <7 and HbA1c ≥7 and diabetics with vascular complications v/s without vascular complications.

Results: MPV, is significantly higher in patients with type -2 diabetes mellitus with HbA1C ≥7 and those with vascular complications in comparison to patients having HbA1c <7 and those without vascular complications (p-value <0.001 which is highly significant).

Conclusions: MPV might be used as a simple and cost-effective laboratory test in the follow up of diabetes mellitus along with HbA1c and thereby help to reduce the morbidity and mortality.

Keywords: Diabetic mellitus, Mean platelet volume, Vascular complications

INTRODUCTION

Diabetes Mellitus is a global pandemic disease. It is a chronic metabolic syndrome principally characterized by persistent hyperglycemic.¹ Impaired fasting glucose is probably a frequent glycemic disorder in the general population and is considered as a pre-diabetic state.² As of 2014 estimated 387 million people have diabetes worldwide.³ Diabetes caused 4.9 million deaths in 2014. Every seven seconds a person dies from diabetes with type 2 diabetes making up about 90% of the cases. The incidence of DM is increasing, and will be more than doubled within 15 years mainly due to adverse life style changes with excess in caloric intake and reduced physical activity.⁴ This is equal to 8.3% of the adult

population, with equal rates in both men and women. Mostly involve people with an age group of 40 to 59 years. Diabetes at least doubles the risk of death.⁵ India is having highest burden of the diabetic subjects. A majority of patients with type 2 DM, as well as subjects with IGT, have signs of the metabolic syndrome (also called dysmetabolic syndrome, insulin resistance syndrome or syndrome X), which is a cluster of phenotypes associated with a substantially increased risk for cardiovascular disease (CVD). Insulin resistance plays a central role in this syndrome. Other components are centripetal obesity, hypertension, dyslipidemia (low HDL, high TG, small dense oxidized LDL) and endothelial dysfunction (microalbuminuria).⁶ Recently, a prothrombotic state, characterized by abnormalities in platelet function and

elevated circulating levels of C-reactive protein (CRP), PAI-1 and fibrinogen, has been recognized as a component of the metabolic syndrome. Microvascular complications (retinopathy, nephropathy and neuropathy) contribute importantly to the increased morbidity in DM as retinopathy and nephropathy are major causes of blindness and end-stage renal disease, respectively. However, the major cause of morbidity and mortality in DM is macrovascular complications. More than 75% of all diabetic patients die of CVD. Insulin resistance, IGT and overt type 2 DM are associated with an increased risk for CVD and patients with type 2 DM have a 2-4 fold increased risk for coronary artery disease and peripheral arterial disease, and a 3-fold increased risk for stroke compared to non-diabetic subjects.⁷

Diabetes also worsens early and late outcomes in acute coronary syndromes and after coronary interventions. There is now consensus that patients with DM without previous myocardial infarction should be treated with multifactorial interventions against modifiable risk factors as aggressively as in non-diabetic individuals with a previous myocardial infarction.⁸ Although type 2 DM is associated risk factors, the exact causes of the substantially eased risk of suffering CVD are not fully understood. Premature, accelerated macrovascular disease occurs both in type 1 and type 2 DM. Recent epidemiological studies indicate that type 1 DM is as great a risk factor for cardiovascular mortality and stroke as type 2 DM, and that these complications also can occur at a young age.⁹

Platelet function is of pathophysiological importance in atherothrombotic disease and there is strong support for platelet dysfunction with platelet hyper reactivity in both type 1 and type 2 DM.^{10,11} It may be hypothesized that platelets, acting in concert with the vascular endothelium, leukocytes and coagulation, play a key role in the development of diabetic angiopathy. While platelet dysfunction is clearly involved in the pathogenesis of macro angiopathy, the role of platelets in microangiopathy is less clear.¹² The metabolic state that accompanies DM may alter platelet and endothelial function already in early stages of diabetic disease. However, it is debatable whether antidiabetic treatment and improved metabolic control can restore the observed platelet hyperactivity in DM. In addition, studies of the effect of acute hyperglycemia on platelet function in patients with DM are sparse.¹³

The increased platelet activity is emphasized to play a role in the development of vascular complications of the metabolic disorder. Large platelets are hemostatically more active and are a risk factor for developing coronary thrombosis, leading to myocardial infarction.¹⁴ The prevalence of diabetic micro-vascular complication is higher in people with poor glycemic control and with long duration of diabetes mellitus.¹⁵ An increased platelet activity has been reported in diabetics as demonstrated by increase in GPIIb/IIIa, Ib-IX and Ia/IIIa.¹⁶ CD62 and CD

63 with increasing availability of blood cell analyzers related to platelets are being estimated most important parameters among them are platelet count, platelet distribution width / and mean platelet volume, the most commonly used measure of platelet size is a potential markers of platelet reactivity.¹⁷⁻¹⁹ It's a new emerging risk factor for atherosclerosis.²⁰ Patient with larger platelets can be easily identified during routine hematological analysis and could possibly benefit from the treatment for predicting the possibility of impending acute events.

The average amount of sugar in the blood can be determined by measuring HbA1c level. It is an important measure of control of diabetes over the last 3 months. Studies on the role of HbA1c indicate that, it reflects the average blood sugar concentration for an extended time period and that it remains unaffected by the short term fluctuation in blood sugar levels. The determination of HbA1c levels serve as a convenient and suitable test for evaluation of the adequacy of diabetic control in the prevention of various diabetic complications. It is sensitive and a specific test for detecting undiagnosed diabetes and at risk individuals or early diabetics.

The risk of diabetic complications, such as diabetic nephropathy and retinopathy, increases with poor metabolic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the development of diabetic complications in diabetes patients.

METHODS

This comparative prospective study was conducted at the J.K Hospital associated with L.N Medical College and research centre, Bhopal during the period of one year. Diagnosis of diabetic patient was established using 2014 ADA Criteria.²¹

- A fasting plasma glucose of >126 mg/dl were considered as diabetics.
- HbA1C \geq 6.5% (48mmol/mol) group.

The totals of 100 subjects were enrolled in the study. The diabetic group, was divided into two namely HbA1c<7 and HbA1c \geq 7 and diabetics with and without vascular complications respectively. Sample for glucose estimation and platelet indices were collected in sodium fluoride and tri-potassium salt of EDTA respectively. Glucose estimation was carried out by the auto-analyzers using enzymatic hexokinase oxidation reference method for plasma glucose levels. Estimation of HbA1C was done using auto analyzer (COBAS C III) which is based on turbidimetric inhibition immunoassay for hemolyzed whole blood. where as platelet indices were done by collecting venous blood samples for complete blood count using automated blood cell count analyzers (NIHON KOHDEN). All test were conducted within 1 hour of sample collection. Informed consent was taken

from all the subjects. 2 ml blood was collected in each vial under aseptic precautions. All consenting non-diabetic subjects were included as control group. Patient already diagnosed of type 2 diabetes and patient who came for routine check-up and were found to diabetes. Exclusion criteria includes subjects having anemia (<13 gm% in males) and (12 gm% in females), malignancy, chronic renal failure, cyanotic heart disease, inflammatory conditions (rheumatoid arthritis, S.L.E), thrombocytopenia, hypo/hyperthyroidism, diabetic on anti-platelet drugs such as aspirin and clopidogrel.

Statistical analysis

The statistical software namely statistical package for the social sciences (SPSS) version 22 is used for analysis of data. Analysis of variance (ANOVA) is used to compare the variables. Data is expressed as mean±standard deviation. The p-value was calculated for each parameter

and p<0.05 is considered statistically significant. Student t-test was used for doing comparison between two variables namely HbA1c <7 v/s HbA1c ≥7 and diabetics with vascular complications v/s without vascular complications. Bar diagram were used for graphical representation of this data.

RESULTS

Diabetic group was also divided into two groups on the bases of HbA1C value. Group 1 with HbA1C level less than 7% and Group 2 with HbA1C level More than 7 %.Out of 100 patients in the diabetic group 10 patients showed HbA1c value to be less than 7 % and 90 patients showed HbA1C to be more than 7%. Fasting plasma glucose was significantly raised in group 2 with HbA1C ≥7%. FPG in group 1 and 2 were 142.55±5.31 mg/dl and 177±22.27 mg/dl respectively, which was highly significant between the two groups with p-value 0.000.

Table 1: Comparing the various parameters in two groups of diabetic patients with reference to HbA1c level.

Parameters	HbA1C less than 7(%)	HbA1C more than 7 (%)	p-value	t-value	Statistical Significance
	10	90			
Mean fasting plasma glucose (mg/dl)	142.55±5.31	177.15±22.27	<0.0001	4.87	S
Mean platelet count (lacs/cumm)	2.44±0.65	2.70±0.79	0.3187	1.002	NS
Platelet distribution width (%)	17.2±0.80	18.6±10.35	0.0018	3.21	NS
Mean of mean platelet volume (fl)	7.87±0.540	9.06±1.72	0.0032	2.17	S
Duration of diabetes (Yrs)	6.4±3.39	6.36±3.68	0.970	0.033	NS

All the platelet indices (platelet count, MPV and PDW) were found to be raised in group 2 with HbA1C ≥7 %. But value of MPV between the two groups shows statistically significant difference in their value with p value<0.0001.

However, PDW and platelet count does not show any significance between two groups with p-value 0.3187 and 0.0018 respectively.

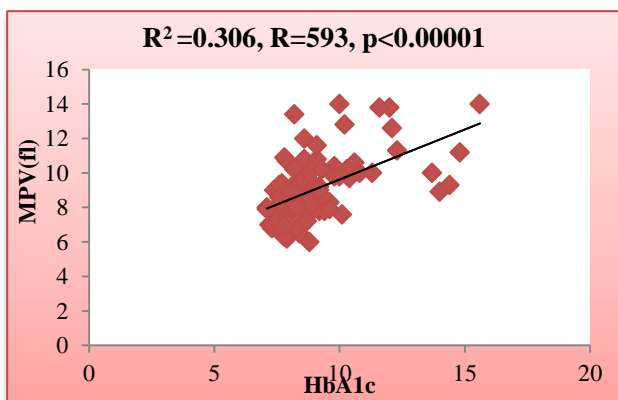


Figure 1: Scatter plot showing positive correlation between HbA1c and mean platelet volume (MPV).

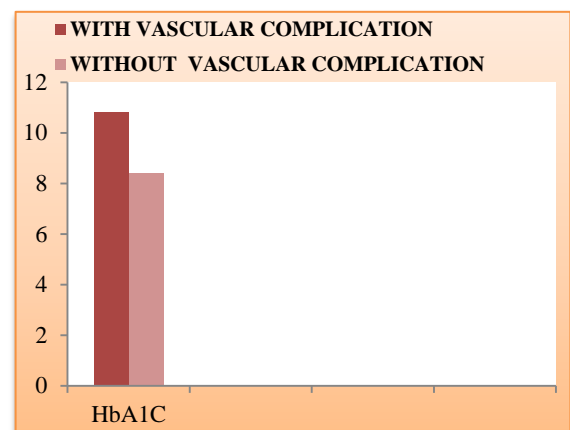


Figure 2: HbA1C of diabetic group with and without vascular complications. HbA1C was also found to be raised in diabetics with vascular complications.

Platelet count in diabetics with complication was found to be 2.86 ± 0.76 lac/cumm in comparison to the platelet count in diabetics without complications which was 2.61 ± 0.78 lac/cumm.

Though the count is slightly high in group with complication, two Groups shows no significant difference in the value of the platelet count. ($p = 0.160$ and $t = 1.41$).

Table 2: Comparisons of glycemc characteristics of diabetic patients with vascular complications and without vascular complications.

Parameters	With diabetic vascular complication N=26	Without diabetic vascular complication N=74	p-value	t-value	Significance
FPG (mean±SD) mg/dl	190.80±24.21	168.51±20.39	<0.0001	4.56	S
HbA1C (%)	10.81±1.81	8.39±1.23	<0.0001	7.58	S

Value of mean Platelet Distribution width (PDW) does not show much difference in the two groups. And statistically they are not significant ($p=0.094$ and $t=1.69$).

Table 3: Comparison of platelet indices in diabetic patient with vascular complications and without vascular complications.

Parameters	With vascular complication	Without vascular complication	p-value	t-value
Platelet count*	2.86 ± 0.76	2.61 ± 0.78	0.160	1.41
PDW	17.93 ± 0.81	18.70 ± 11.55	0.094	1.69
MPV	10.62 ± 2.13	8.40 ± 1.00	<0.0001	7.06
N	26	74		

*(LAC/CUMM)

The Mean platelet volume is 10.62 ± 2.13 fl in diabetic group with vascular complications which is found to be raised in comparison to the group without vascular complications which is statistically significant with p value <0.0001(t-value 7.06).

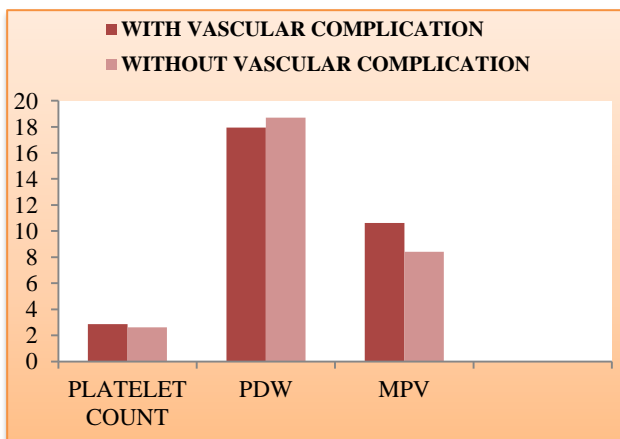


Figure 3: Comparison of mean platelet parameters with complication and without complication in the diabetic group.

DISCUSSION

The mean fasting plasma glucose level was found to be 190.80 ± 24.21 mg/dl in diabetic patient with vascular complications and fasting plasma glucose level in diabetics without complication was found to be 168.51 ± 20.39 mg/dl. This shows that FPG was slightly higher in group with vascular complications. Which is statistically significant with p value <0.0001(t-value 7.06).

The Mean platelet volume is 10.62 ± 2.13 fl in diabetic group with vascular complications which is found to be raised in comparison to the group without vascular complications.

HbA1C was also found to be raised in diabetics with vascular complications in comparison with diabetics without vascular complications. HbA1C was $10.81 \pm 1.81\%$ and $8.39 \pm 1.23\%$ respectively in the diabetic group with or without vascular complications. This difference in the value is significant ($p < 0.0001$). Li S et al showed that the MPV level was highest in the type-2 DM group (12.3 ± 1.5) fl.²² It was significantly higher in diabetics with HbA1c $\geq 7\%$ (13.2 ± 1.9) fl than in patients those with HbA1c $< 7\%$ (11.8 ± 1.7) fl ($p < 0.01$). Multiple Logistic regression analysis indicated that MPV was an important risk factor of peripheral arterial disease.

Demirtune R et al showed that MPV was significantly higher in patients with DM than in controls (8.7 ± 0.8 fl vs. 8.2 ± 0.7 fl, $p = .002$).² In diabetic patients, with vascular complications there was a significant positive correlation between MPV and HbA1c levels ($r = 0.39$, $P = .001$) but not in the diabetic group without vascular complications. When they compared the two diabetic groups, Group B (HbA1c > 7) patients had significantly higher MPV than Group A (HbA1C < 7) (9.0 ± 0.7 fl vs. 8.4 ± 0.8 fl, $p = 0.01$)

If vascular damage was only due to increased number of large and reactive platelets, then the rate of damage would have been constant for the duration of disease and independent of diabetic control. This clearly shows that

Platelet reactivity alone cannot explain the progression of vascular complication in diabetes mellitus, since there are other vascular risk factors that may be influenced by degree of control of diabetes. This is supported by non-significant statistical correlation between MPV and duration of diabetes in our study. Microvascular complications (retinopathy, nephropathy and neuropathy) contribute importantly to the increased morbidity in DM as retinopathy and nephropathy are major causes of blindness and end-stage renal disease, respectively. However, the major cause of morbidity and mortality in DM is macrovascular complications. More than 75% of all diabetic patients die of CVD. Insulin resistance, IGT and overt type 2 DM are associated with an increased risk for CVD and patients with type 2 DM have a 2-4 fold increased risk for coronary artery disease and peripheral arterial disease, and a 3-fold increased risk for stroke compared to non-diabetic subjects. Diabetes also worsens early and late outcomes in acute coronary syndromes and after coronary interventions.^{6,7}

Platelet in diabetes mellitus have dysregulated signaling pathway that lead to an increased activation and aggregation in response to a given stimulus, thus triggering thrombus formation and causing micropapillary embolization with release of constrictive, oxidative and mitogenic substances such as PDGF and VEGF that accelerate progression of local vascular lesion like the neovascularization of lens in diabetic retinopathies.²³ Diabetic group was also divided into two groups on the bases of HbA1C value. Group 1 with HbA1C level less than 7% and Group 2 with HbA1C level More than 7%. Out of 100 patients in the diabetic group 10 patients showed HbA1c value to be less than 7% and 90 patients showed HbA1C to be more than 7%. Fasting plasma glucose was significantly raised in group 2 with HbA1C \geq 7%. FPG in group 1 and 2 were 142.55 \pm 5.31 mg/dl and 177 \pm 22.27 mg/dl respectively, which was highly significant between the two groups with p-value 0.0001. All the platelet indices (platelet count, MPV and PDW) were found to be raised in group 2 with HbA1C \geq 7%. But value of MPV between the two groups shows statistically significant difference in their value with p value <0.0001. However, PDW and platelet count does not show any significance between two groups with p-value 0.3187 and 0.0018 respectively.

Ozder A also compared the platelet count between the two groups and does not found much difference between the two groups.²⁴ The mean Platelet count in two groups were 242.66 \pm 57.30 and 254.98 \pm 74.66 with p value 0.339 which was not significant. Earlier studies have emphasized that HbA1c is specific sensitive and significant method for estimation of glycemic levels. HbA1c increases with poor glycemic control. Other studies have patients distribution on the basis of HbA1c where Gresesle et al (2003), Farah Jabeen et al (2013) and Kodiatte et al (2012).^{1,25,26} However, these authors compared only two groups i.e control and diabetic and

therefore there finding were limited as compared to a study which includes pre diabetic patients (I.F.G).

There was also a significant association between HbA1c and MPV, Which was again seen in study done by Demirtune et al.² Therefore, it may be concluded that glycemic control improves platelet activity and function and may prevent or delay the possible diabetic vascular complications. Study done by Kodiatte TA et al showed the similar finding that in diabetes mellitus mean platelet volume (MPV) is increased and had a positive correlation with HbA1c Ulutas KT et al (2014).^{1,27} In this study, all diabetic patients were divided into two groups according to their HbA1c levels: group A consisted of patients with HbA1c levels \leq 7% and group B consisted of patients with HbA1c levels $>$ 7%. Concluding that MPV was significantly higher in Group B as compared to both non-diabetics and Group A. MPV had a high positive correlation with HbA1c and FSG, as with diabetes duration. It is found that MPV was increased in type 2 DM. Ozder found that patient with diabetes and subjects with IFG, MPV was significantly higher (10.66 \pm 0.94 fl and 10.49 \pm 0.96 fl, respectively) as compared to the non-diabetic group (10.04 \pm 1.04 fl) (p=0.000).²⁴ Among the diabetic group, a positive statistical correlation Pearson correlation was seen between MPV and HbA1c levels (r=0.357; p=0.000) and fasting blood glucose levels (r=0.306; p=0.0000). The mean MPV in patients having HbA1c <7.5 % was 10.17 \pm 0.83 fl and significantly lower than that of patients with HbA1c \geq 7.5 % (10.30 \pm 0.92 fl) (p=0.001). Our findings suggested an association between MPV and HbA1c. Therefore, MPV would be a beneficial prognostic marker of cardio-vascular complications.

CONCLUSION

Most of the patient with type II DM suffers from preventable vascular angiopathies and early diagnosis of progressive activation of coagulation can help manage these vascular diseases successfully. Therefore, MPV and HbA1c can be a useful as prognostic marker of cardiovascular complication in diabetics. MPV might be used as a simple and cost – effective laboratory test in the follow up of DM along with HbA1c and thereby help to reduce the morbidity and mortality.

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

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