ORIGINAL ARTICLE

Study of Impact of Glycemic Status (HbA1c) on Platelet Activity measured by Mean Platelet Volume & Vascular Complications in Diabetics

Manoj Saluja[1], Yogesh Kumar Swami[2*], SR Meena[3]

Abstract

Introduction: Diabetes mellitus is a global pandemic. The increased platelet activity may play a role in the development of vascular complications of this metabolic disorder. The mean platelet volume (MPV) is an indicator of the average size and activity of platelets. Larger platelets are younger and exhibit more activity.

Aims and Objectives: To determine the MPV in diabetics with different glycemic control (HbA1C), to see if there is a difference in MPV between diabetics with and without vascular complications, and to determine the correlation of MPV with fasting blood glucose, glycosylated hemoglobin (HbA1c), body-mass index, and duration of diabetes in the diabetic patients.

Methodology: Platelet counts and MPV were measured in 160 Type 2 diabetic patients using an automated blood cell counter. The blood glucose levels and HbA1c levels were also measured. All patients were divided in 2 groups, group A, which includes patients with HbA1C≤8 % and group B, which includes patients with HbA1C>8 %. Statistical evaluation was performed using Student's t test and Pearson correlation tests

Results: The mean platelet counts and MPV were higher in diabetics with higher HbA1C (group B) compared to the diabetics with lower HbA1C (group A) [288.30 ± 103.96 X 109/l vs. 265.83 ± 66.97 X 109/l (*P*= 0.16)], 13.77 ± 0.08 fl versus 11.86 ± 0.66 fl (*P*= 0.0001), respectively. MPV showed a positive correlation with fasting blood glucose (regression (r) = 0.18) and HbA1C levels (*P*=0.0001). HbA1C and MPV increases with increase in duration of DM, which were 8.62±0.96 and 8.51±1.09 % (p=0.49) and 13.24±1.27 and 13.10±1.37 (p=0.50) respectively in both group with duration >5 years and ≤5 years. On the basis of vascular complications, HbA1C, MPV and Duration of DM were (in both group with and without complications respectively), 8.58±0.01 % and 8.56±0.09 % (p=0.03), 13.12±1.40 fl and 12.80±1.21fl (p=0.13), 9.11±3.22 years and 2.5±2.2 years (p<0.0001).

Conclusion: Our results showed significantly higher MPV in diabetic patients with higher HbA1C (poor glycemic control). This indicates that elevated MPV could be either the cause for or due to the effect of the vascular complications. Hence, platelets may play a role and MPV can be used as a simple parameter to assess the vascular events in diabetes.

Introduction

Diabetes mellitus (DM) is a major global health problem. According to estimates of the World Health Organisation, the number of people with DM has risen from 108 million in 1980 to 422 million in 2014 there.[1]

The increased platelet activity is emphasized to play a role in the development of vascular complications of this metabolic disorder.[2] Platelet volume, a marker of the platelet function and activation, is measured as mean platelet volume (MPV) by hematology analyzers. Diabetic patients have an increased risk of developing micro- and macrovascular disease, and platelets may be involved as a causative agent with respect to altered platelet morphology and function.[3,4]

The aim of our study was to determine if platelets were activated in diabetes and in its associated vascular complications by measuring the MPV in the diabetics, to see if there was a difference in MPV in diabetics with and without vascular complications, and to determine the correlation of MPV with fasting blood glucose (FBS), postprandial plasma glucose (PPBS), glycosylated hemoglobin (HbA1c), body-mass index (BMI), and duration of diabetes in the diabetic patients, respectively.

Materials and Methods

Study design

This was a cross sectional study carried out in 160 patients who were already diagnosed to have Type 2 DM. All patients underwent a complete clinical evaluation with specific reference to any associated macro- or microvascular complications. Height and weight of all the subjects were recorded. We measured the MPV and platelet counts with complete blood count using an automatic blood counter (Beckman Coulter Act5Diff). The estimation of plasma glucose levels (fasting plasma glucose and postprandial plasma glucose) was carried out by the glucose oxidase method in the auto analyzer (Johnson and Johnson vitros 250) and that of HbA1c by the high-performance liquid chromatography method.

Inclusion Criteria

1. Diabetic patient diagnosed according to ADA Criteria.

[1]Professor, [2]Resident, [3]Senior Professor, Govt. Medical College, Kota, Rajasthan; *Corresponding Author
Received: 04.05.2018; Revised: 11.01.2019; Accepted: 20.03.2019

Table 1: Various parameters studied in study population

Parameter	Value/No./%
No of patients	160
Age	51.9±13.5 years
Males	84
Females	76
Mean duration of DM	5.97±4.33 years
Macro and micro vascular complications (no. of patients)	96 (60%)
BMI	24.04±3.26 kg/m^2
FBS	151.32±34.25 mg/dl
HbA1C	8.57±0.01%
Platelet count	281562±94539
MPV	13.17±1.31 fl

a. HbA1C ≥ 6.5% (Lab NGSP certified standardized assay) ,or

b. Fasting glucose ≥ 126 mg/dl (no caloric intake for at least 8 hours), or

c. 2 hour glucose ≥200 mg/dl (after 75 gm of anhydrous glucose), or

d. Random glucose ≥ 200 mg/dl (plus classic hyperglycemia symptoms).

Exclusion Criteria

1. Abnormal platelet count(<100 and >400×10^3/μL)
2. Use of drugs affecting platelet function (aspirin, warfarin, ticlopidine, or heparin)
3. Using statin therapy
4. Male patients with Hb<12mg/dl and females with Hb<10 mg/dl
5. Pregnant females
6. Patients with malignancy

After baseline evaluation, diabetic patients were divided into two groups according to their HbA1c levels: group A consisted of patients with HbA1c levels ≤ 8% and group B consisted of patients with HbA1c levels > 8%.

Data collection

All patients who fulfilled the inclusion/exclusion criteria selected from the inpatients and outpatients departments of New Medical College Hospital and MBS Hospital Kota. A detailed history including physical examination was done on all subjects to rule out other compounding causes and factors affecting MPV.

Statistical analysis

Statistical evaluation was performed using Student's independent sample two-tailed t-test and Pearson correlation test (*r* value as the coefficient). Data were expressed as mean ± standard deviation. A *P* value <0.05 was considered statistically significant.

Table 2: Correlation of MPV to the different parameters studied

Parameters		r value	p value
MPV	Duration of DM	0.2	0.72
MPV	BMI	0.72	**0.02**
MPV	HbA1C	0.9	**<0.0001**
MPV	FBS	0.64	**0.03**
MPV	Complications	-	0.13
MPV	Age	-0.26	**0.03**

Results

Subject population

Total 160 diabetic subjects were included in this study of which 84 were males and 76 were females. The mean age of the study population was 51.9±13.5 years. Of all 3 age groups (20-39, 40-59 and ≥60 years of age), 36, 80 and 44 subjects were included respectively. The mean duration of diabetes was 5.97±4.33 years (patients were studied in 2 groups as duration ≤5 and >5 years). All subjects were divided in 3 groups on the basis of BMI (18.5-24.9, 25-29.9, ≥30 kg/m^2), each included 36, 80 and 44 respectively.

All diabetic subjects in this study were divided in 2 groups on the basis of HbA1C, group A (HbA1C≤8 %) and group B (HbA1C>8%).

Observation and Results

Out of the 160 diabetics, 96 (60%) had sign and symptoms of complications such as peripheral neuropathy, diabetic foot, diabetic retinopathy, diabetic nephropathy, hypertension, coronary artery disease, peripheral vascular disease and 64(40%) did not have any of these complications.

The mean BMI in the study population was 24.04±3.26 kg/m^2 (It was 25.2±1.83 kg/m^2 in patients with HbA1C≤8 was and 28.3±3.51 kg/m^2 in patients with HbA1C>8.)

Among the diabetic subjects, a positive statistical Pearson correlation was seen between MPV and HbA1c levels (r = 0.9; P < 0.0001), FBS levels (r = 0.64; P < 0.03), BMI (r =0.72, p =0.02). However, no statistical correlation was seen between MPV and the duration of DM (p=0.50) and the vascular complications (p=0.13) in the diabetic group.

The mean MPV in subjects with complications (13.12±1.40 fl) was higher than that of subjects without complications (12.80±1.21 fl) but independent student t-test did not show any statistical significance (P = 0.13).

Table 3: Comparative study of different parameters in group A and B

Characteristic	Group A	Group B	P value
No of patients	48	112	-
MPV(fl)	11.86±0.66	13.77±1.08	0.0001
HbA1C (%)	7.44±0.03	9.06±0.08	0.0001
BMI (kg/m^2)	25.2±1.83	28.3±3.51	0.0001
Platelet count (× 10^9/L)	265.8±66.9	288.3±103.9	0.16
FBS (mg/dl)	120.6±15.2	164.4±31.6	<0.001

Out of 160 DM patients, there were 48 patients in group A (mean HbA1c 7.44±0.03%) and 112 patients in group B (mean HbA1c = 9.06±0.08%). The mean BMI in group A (25.2±1.83 kg/m^2) was significantly lower than that of group B (28.3±3.51 kg/m^2; P = 0.0001). The mean FBS level in group A was 120.6±15.2 mg/dL while that of group B was 164.4±31.6 mg/dL(P < 0.001). The mean platelet count in group A (265.8±66.9 × 10^9/L) was higher than that of group B (288.3±103.9 × 10^9/L) but was not statistically significant (p=0.16). The mean MPV in group A (11.86±0.66 fl) was significantly lower than that of group B (13.77±1.08 fl; P = 0.0001).

Mean HbA1C in patients with duration of DM >5 years was 8.62±0.96 and in patients with duration ≤5 years it was 8.51±1.09 (p=0.49). Glycemic control improves with age, as mean HbA1C in group with age>50 years was 8.27±0.009 and in group age≤50 years it was 8.95±0.009 (p<0.001). MPV also decreases with age, as it was13.51±1.21% in age group ≤50yearsand 12.9±1.35% in age group >50 years. (p=0.03)

Discussion

DM is a complex metabolic syndrome characterized by chronic hyperglycemia resulting in complications affecting the peripheral nerves, kidneys, eyes, and micro- and macrovascular structures.[2] The prevalence of all types of diagnosed diabetes in most western societies is 3–7%. Countries with the highest absolute number of diabetics are in India (19 million), China (16 million), and the United States (14 million). The prevalence of diabetic microvascular complications is higher in people with poor glycemic control, longer duration of DM.[4] Diabetes and its vascular

complications can cause a financial burden to a country's national economy. India, having the highest number of diabetics, faces such issues. MPV can be used as a simple economical test in the monitoring of DM and thereby help curb the morbidity and mortality.

Type 2 DM is characterized mainly by impaired insulin secretion and increased tissue insulin resistance.[2] Sustained hyperglycemia leads to a series of interrelated alterations that can cause evident endothelial dysfunction and vascular lesions in diabetic complications.[5] Formation of advanced glycation end products, activation of protein kinase C and disturbances in polyol pathways are the possible mechanisms by which increased glucose induces vascular abnormalities.[6]

Platelets are small discoid blood cells that circulate and participate in hemostasis. Primary plug formation due to platelets seals the vascular defects and provides the required phospholipid surface for the recruited and activated coagulation factors.[7] In response to stimuli generated by the endothelium of blood vessels, platelets change shape, adhere to subendothelial surfaces, secrete the contents of intracellular organelles, and aggregate to form a thrombus.[7] These pro-aggregatory stimuli include thrombin, collagen, epinephrine, ADP (dense storage granules), and thromboxane A2 (activated platelets).[7] Thus, platelets may assume an important role in signaling of the development of advanced atherosclerosis in diabetes.[5,7-9]

MPV is an indicator of the average size and activity of platelets. Larger platelets are younger, more reactive and aggregable. Hence, they contain denser granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets.[7,9-11] All these can produce a pro-coagulant effect and cause thrombotic vascular complications. This suggests a relationship between the platelet function especially MPV and diabetic vascular complications thus indicating changes in MPV reflect the state of thrombogenesis.[3,5] Thus, DM has been considered as a "prothrombotic state" with increased platelet reactivity.[12]

Hyperglycemia can increase platelet reactivity by inducing nonenzymatic glycation of proteins on the surface of the platelet, by the osmotic effect of glucose and activation of protein kinase C.[13-15] Such glycation decreases membrane fluidity and increases the propensity of platelets to activate.[13-15] Platelet function is directly regulated by insulin via a functional insulin receptor (IR) found on human platelets.[13–15] *In vivo* experiments have confirmed that insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonists in healthy nonobese individuals.[13–15]

MPV can also be elevated as an end result of an atherothrombotic event like myocardial infarction. This could be due to the quicker consumption of smaller platelets in the vascular event and compensatory production of reticulated platelets.[16,17]

In our study, the mean platelet count was higher in the diabetic group with higher HbA1C (poor glycemic control) that was similar to the studies done by Demirtunc *et al*.[2] and Zuberi *et al*.[4] Other studies by Hekimsoy *et al*.[3] had observed the opposite finding with lower platelet counts in the diabetic group with lower HbA1C. Hence, the platelet count could be dependent on several variables, that is, mean platelet survival, platelet production rate, and turnover rate in DM.

Higher values of MPV were observed in diabetic subjects with microvascular complications such as retinopathy but were not statistically significant. Higher values were also seen in the studies done by Ates *et al*.[10] and Papanas *et al*.[18] This suggested a role for the increased platelet activity in the pathogenesis of vascular complications. On the other hand, in the studies done by Hekimsoy *et al*. and Demirtunc *et al*. MPV was not significantly different in subjects with diabetic neuropathy/retinopathy from that of diabetics without those complications.[2,3] Their possible explanation was centered on the rapid consumption of activated platelets in diabetics with complications.[2,3]

In our study, MPV was significantly higher in diabetics with HbA1c levels > 8% than in diabetics with HbA1c levels ≤8%. There was a significant association between HbA1c and MPV, which was again seen in the study done by Demirtunc *et al*.[2] Therefore, it may be concluded that glycemic control decreases the hyper activity of the platelet function and thus may prevent or delay possible diabetic vascular complications. However, our data needs to be further confirmed in larger studies. The reason for a high number of diabetics with HbA1c levels > 8% in the current study might have been due to poor dietary practices and lack of knowledge regarding the diet and exercise regimens that ought to be followed in diabetics.

No significant MPV association was seen with duration of diabetes and presence of complications. Similar findings were seen in other studies.[2,3] But our findings were in contrast to the study done by Ates *et al*.[10] Where MPV was positively correlating with the degree of retinopathy in their cases.

Conclusion

In diabetes mellitus, platelets become more reactive and aggregable and their mean volume (MPV) is increased. The increased platelet size may be one factor in the increased risk of atherosclerosis associated with diabetes mellitus and associated vascular complications. Hence, MPV would be a useful prognostic marker of cardio-vascular complications in diabetes. We also found that increase in HbA1c concentration was directly proportional to increased MPV. However, the increased MPV as the cause or the end result of vascular complications needs to be further explored. Hence, we propose that MPV can be used as a simple and cost-effective tool to monitor the progression and control of DM and its cardio-vascular complications.

References

1. World Health Organization August 2011. Last accessed on 2011 Nov 17. Available from: http://www.who.int/mediacentre/factsheets/fs312/en/.
2. Demirtunc R, Duman D, Basar M, Bilgi M, Teomete M, Garip T, The relationship between glycemic control and platelet activity in type 2 diabetes mellitus. *J Diabetes Complications* 2009; 23:89–94.
3. Hekimsoy Z, Payzinb B, Ornek T, Kandogan G, Mean platelet volume in Type 2 diabetic patients. *J Diabetes Complications* 2004; 18:173–6.
4. Zuberi BF, Akhtar N, Afsar S, Comparison of mean platelet volume in patients with diabetes mellitus, impaired fasting glucose and non-diabetic subjects. *Singapore Med J* 2008; 49:114–6.
5. Bae SH, Lee J, Roh KH, Kim J, Platelet activation in patients with diabetic retinopathy. *Korean J Ophthalmol* 2003; 17:140–4.
6. Maitra A; Kumar V, Abbas AK, Fausto N, Aster JC, The Endocrine System. Robbins and Cotran Pathologic Basis of Disease. 2010. 8th ed. p. 1097–164
7. Mitchell RN, Kumar V, Abbas AK, Fausto N, Aster JC. Hemodynamic Disorders, Thromboembolic Disease and Shock. Robbins and Cotran Pathologic Basis of Disease.

2010. 8th ed; p. 111–34

8. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Jimenez-Quevedo P, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005; 54:2430–5.
9. Colwell JA, Nesto RW, The platelet in diabetes-focus on prevention of ischemic events. *Diabetes Care* 2003; 26:2181–8.
10. Ateş O, Kiki I, Bilen H, Keleş M, Koçer I, Kulaçoğlu DN, et al. Association of Mean Platelet Volume With The Degree of Retinopathy in Patients with Diabetes Mellitus. *Eur J Gen Med* 2009; 6:99–102.
11. Chang HA, Hwang HS, Park HK, Chun MY, Sung JY. The Role of Mean Platelet Volume as a Predicting Factor of Asymptomatic Coronary Artery Disease. *Korean J Fam Med* 2010; 31:600–6.
12. Jindal S, Gupta S, Gupta R, Kakkar A, Singh HV, Gupta K, et al. Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications. *Hematology* 2011; 16:86–9.
13. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; 24:1476–85.
14. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. *Diabetes Care* 2009; 32:525–7.
15. Kakouros N, Rade JJ, Kourliouros A, Resar JR. Platelet function in patients with diabetes mellitus: from a theoretical to a practical perspective. *Int J Endocrinol* 2011; 2011:742719
16. Sewell R, Ibbotson RM, Phillips R, Carson P. High mean platelet volume after myocardial infarction: is it due to consumption of small platelets? *Br Med J (Clin Res Ed)* 1984; 289:1576–8.
17. Boos CJ, Lip GY. Assessment of mean platelet volume in coronary artery disease- what does it mean? *Thromb Res* 2007; 120:11–3.
18. Papanas N, Symeonidis G, Maltezos E, Mavridis G, Karavageli E, Vosnakidis T, et al. Mean platelet volume in patients with type 2 diabetes mellitus. *Platelets* 2004; 15:475–8.