ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 14, Issue, 03, pp.21002-21005, March, 2022

DOI: https://doi.org/10.24941/ijcr.43269.03.2022

RESEARCH ARTICLE

A CASE CONTROL STUDY ON RELATION BETWEEN THE VARIATIONS IN PLATELET COUNTS AND MEAN PLATELET VOLUME IN TYPE 2 DIABETIC PATIENTS ON TREATMENT AND NON-DIABETIC CONTROLS

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ARTICLE INFO

Article History:
Received 24th December, 2021
Received in revised form
19th January, 2022
Accepted 24th February, 2022
Published online 30th March, 2022

Keywords:

Diabetes Mellitus, Mean Platelet Volume, Mean Platelet Count.

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ABSTRACT

Background: It is observed that in diabetes there is an enhanced platelets activation and increase in coagulation proteins and fibrinolytic activity is reduced. These are the pro thrombotic states which further lead to the development of cardiovascular and atherosclerotic complications. Studies have indicated that the patients of Type 2 DM have two to four folds increase in risk of atherosclerosis. Mean Platelet Volume (MPV), which is the average volume of platelets and measures platelet size distribution, and is not influenced by glycemic control. Studies have shown that a higher incidence of proliferative diabetic retinopathy and myocardial infarction are associated with increased MPV. It is observed in the diabetics that larger than normal platelets are in circulation, this is due to the activated megakaryocyte-platelet system. Platelet count and MPV can prove to be an inexpensive, simple and effective tests that may be used to predict angiopathy in type 2 DM. Few studies have used Elevated MPV levels to predict bad outcome for acute ischemic cerebrovascular events independent of other clinical parameters. **Aims and Objectives**: To study the relation between the variations in platelet counts and mean platelet volume in type 2 diabetic patients on treatment and non-diabetic controls. **Methods:** Acase control study. **Results**: The overall mean platelet volume was 8.41±0.68fl, for the diabetics 8.72±0.71 fl and the non-diabetic controls 8.94±0.79 fl.There was a statistically significant difference in platelet count of diabetics and healthy controls platelet counts. p =0.049 and t value of 1.99. There was no statistically significant difference between the mean platelet volume in diabetics and healthy controlswith p=0.146 and t value of 1.46. Positive correlation was observed between MPV and fasting blood sugar (r = 0.027, p <0.001) when Pearson's correlation test was applied.Also, Body Mass Index (r =0.147) and duration of diabetics (r =0.026) were positively correlated.However, platelet count and fasting blood sugar were negatively co related (r = -0.048),Platelet count was also negatively correlated with duration of diabetics (r = -0.021). Platelet count and body mass index (r = 0.032) were positively correlated. In both the diabetic patients and non-diabetic controls the relation between the platelet count and mean platelet volume showed positive correlation, Pearson's test was used and it showed statistically significant levels of 0.041 in both groups. **Conclusion**: The present study showed that the diabetics on treatment had higher mean platelet count than that of non-diabetics controls, it was also noted that mean platelet volume was lower in controls than in cases.

Citation: Dr. Tanvi Rajan Prabhu and Dr. Jayaraj R Mhetri. *"*A case control study on relation between the variations in platelet counts and mean platelet volume in type 2 diabetic patients on treatment and non-diabetic controls", *2022. International Journal of Current Research, 14, (03), 21002-21005.*

INTRODUCTION

Type 2 Diabetes mellitus make up majority of Diabetic population, 80 % of the diabetes patients are of Type 2 Diabetes mellitus (DM) (Ostenson, 2001). Multiple factors are responsible for the evolution of diabetes, interaction between environmental and genetic factors have an important role in development of type 2 DM (Ostenson, 2001).

It is observed that in diabetesthere is an enhanced platelets activation and increase in coagulation proteins and fibrinolytic activity is reduced (Carr, 2001). These are the pro thrombotic states which further lead to the development of cardiovascular and atherosclerotic complications (Mendel, 1993). Studies have indicated that the patients of Type 2 DM have two to four folds increase in risk of atherosclerosis (Beckman *et al*., 2008). In a study done by Luscher *et al*. (2003) it was observed that there is increased risk of cardio vascular event as a result of

accelerated atherosclerosis in DM. The platelet function has an important role in pathophysiology in evolution of atherothrombosis, especially in diabetic patients (Colwell, 2003) Multiple studies have concluded that macro vascular complications in diabetes are due to platelet dysfunction, which in turn lead to higher incidence of morbidity and mortality in type 2 DM (Ferroni, 2004; Ishii, 1992; Vinik, 2001). Also, an increase in GPs IIb/IIIa, 1b-IX, and 1a/IIa in diabetics show that platelet activity is higher in diabetics (Tschoepe, 1990), Mean Platelet Volume (MPV), which is the average volume of platelets and measures platelet size distribution, and is not influenced by glycaemic control (Sharpe, 1993). Studies have shown that a higher incidence of proliferative diabetic retinopathy (Tschoepe, 1995) and myocardial infarction (Martin, 1991) are associated with increased MPV. It is observed in the diabetics that larger than normal platelets are in circulation, this is due to the activated megakaryocyte-platelet system (Sharpe, 1993). Platelet count and MPV can prove to be aninexpensive, simple and effective tests that may be used to predict angiopathy in type 2 DM. few studies have used Elevated MPV levels to predict bad outcome for acute ischemic cerebrovascular events independent of other clinical parameters (Lalouschek, 2001). The present study aims to study the relation between the variations in platelet counts and mean platelet volume in type 2 diabetic patients on treatment and non-diabetic controls

METHODS

Study Population: The present study was aconducted in hundred patients of type 2 diabetic patients on treatment when came for the testing of diabetic profile at laboratory.The study was conducted for a period of 3 months, all the patients who gave informed consent and satisfied the study inclusion criteria were recruited into the study.

Study Design: The present study is an unmatched case control study, a total of 100 study participants were divided into the 50 cases who were proved diabetics and 50 non-diabetic controls controls.

Data collection: The study subjects were asked to fill structured questionnaires which included socio demographic information, height, weight, last fasting blood sugar, blood pressure, drug history, and family history of diabetes.

Inclusion Criteria for the cases: All non-insulin dependent diabetes mellitus patients on treatment attending the diabetes clinic.

Exclusion Criteria for the cases: Non-diabetic patients and insulin-dependent diabetes mellitus patients.

Inclusion Criteria for the controls: All consenting non-diabetics adults.

Exclusion Criteria for the controls: Diabetics adults on oral hypoglycemic drugs.

Sample Collection: Blood specimen was withdrawn with minimal stasis from the ante-cubital vein using a dry sterile disposable syringe and needle. Four and half milliliters of blood was dispensed into EDTA anticoagulant tubes. The specimens were labeled with subject's age, sex and identification number. The EDTA samples were kept at room temperature until processed within four hours of collection.

Laboratory Analysis: Fully automated bidirectional analyzer (6 part differential SYSMEX X N -1000) was used to perform hematology analyses according to the hydrodynamic focusing by DC method , flow cytometry using semiconductor laser and SLS hemoglobin method.Standardization, calibration of instrument and processing of samples were done according to manufacturer's instructions.

Procedure: Well mixed blood sample was aspirated, by letting the equipment sampling probe into the blood sample and then pressing the start button. Approx. 20 microliter of blood was aspirated by the auto analyzer. Result of analysis is displayed after about 30secs.A printout copy of result is released on the thermal printing paper.

Statistical Analysis: Data was entered in Microsoft Excel and analyzed using Open Epi 2.3.1 software, the means ± standard deviation (SD) was used for continuous variables. The Pearson chi squared test was used to test for association between discrete variables. P value was considered to be statistically significant when < 0.05. Students t test was used to compare means between two groups.

RESULTS

The present study consisted of 100 diabetic patients. The mean age of study subjects was 48.25 ± 8.64 years amongst cases and in controls the mean age was 51.35±9.41, this was not statistically significant with t value of 1.71 and p value of 0.089, i.e. the the groups were comparable.The majority of the study participants were male with 56% and 44% of the study participants were women in cases and in controls 52% were males and 48% were females. TheMean BMI of the cases was 30.14± 4.82 in controls were 27.46±5.41 with p value of 0.01, which was statistically significant. The mean fasting blood sugar of diabetics was 149.45±67.54 mg/dl in controls it was 99.64±28.10 mg/dl, this was statistically significant with t value of 4.814 and p value of 0. 0001.Amongstthe cases 48% of the participants gave family history ofdiabetes where as in controls only 13% of the participants gave family history of diabetes. The mean duration of diabetics in the cases was 7.67±6.46 years. In the present study mean platelet count was 226.79 ± 74.51 $*10^9$/L, for the diabetics 238.47±78.41$*10^9$/L and controls, 214.31±40.32$*10^9$/L. (table no 1). The overall mean platelet volume was 8.41±0.68fl, for the diabetics 8.72±0.71 fl and the non-diabetic controls 8.94±0.79 fl.There was a statistically significant difference in platelet count of diabetics and healthy controls platelet counts. p =0.049 and t value of 1.99. There was no statistically significant difference between the mean platelet volume in diabetics and healthy controlswith p=0.146and t value of 1.46. Positive correlation was observed between MPV and fasting blood sugar (r = 0.027, p <0.001) when Pearson's correlation test was applied.(Table no 2) Also, body mass index (r =0.147) and duration of diabetics (r =0.026) were positively correlated.However, platelet count and fasting blood sugar were negatively co related (r = -0.048),Platelet count was also negatively correlated with duration of diabetics (r = -0.021). Platelet count and body mass index (r = 0.032) were positively correlated. In both the diabetic patients and non-diabetic controls the relation between the platelet count and mean platelet volume showed positive correlation,Pearson's test was

used and it showed statistically significant levels of 0.041 in both groups. Table no 1. showing the distribution of gender, family history and comparison of mean age, BMI, fasting blood sugar, platelet count and platelet volume in the two groups

In the present study a positive relationship between the MPV and the duration of diabetes was observed it supports the fact that that the risk of microvascular complications increases with the duration of diabetes.

Unpaired Students t test was used to compare the means between two groups, p value < 0.05 was considered statistically significant

SL NO	PARTICULARS		CASES N=50	CONTROLS N=50	P VALUE	T VALUE
1	Mean age		48.25 ± 8.64	51.35±9.41	0.089	1.712
2	Gender	Male	56%	52%	0.285	0.332*
		Female	44%	48%		
3	Body mass index		30.14± 4.82	27.46±5.41	0.010	2.615
4	Mean Fasting blood sugar		149.45±67.54	99.64±28.10	0.0001	4.814
5	Family History of diabetes	Present	48%	13%	<0.001	28.89*
		Absent	52%	87%		
6	Mean Platelet Count		238.47±78.41*10^9/L	214.31±40.32*10^9/L.	0.049	1.99
7	Mean Platelet Volume		8.72±0.71 fl	8.94±0.79 fl	0.146	1.46

*Chi square tests was used, p value < 0.05 was considered statistically significant

Table no 2. Showing the correlation between the MPV and MPC with FBS, BMI and duration of diabetics

Sl no	Pearson's Correlation	r value
1	MPV and fasting blood sugar	0.027
2	MPV and BMI	0.147
3	MPV and duration of diabetics	0.026
4	Mean platelet count and fasting blood sugar	-0.048
5	Mean platelet count and duration of diabetics	-0.021
6	Platelet count and body mass index	r = 0.032

Pearson's correlation was used to determine the correlation between the variables.

DISCUSSION

The mean platelet volume and platelet counts are potential indicators of thrombotic event; the deranged values are risk factors for microvascular complications in diabetics (Hekimsoy, 2004; Zuberi, 2008; Bae, 2003). An average size and activity of platelets is indicated by MPV, higher MPV value indicatesa larger average platelet size. Higher amounts of thromboxane A2 are synthesized by larger platelets, the aggregation of the platelets isbetter, also they are able to secrete higher amounts of serotonin and β-thromboglobulin than smaller platelets (Colwell, 2003; Ates, 2009; Chang, 2011). In the present study showed that higher mean platelet count was in diabetics than in the controls. Few other studies by Thomas *et al*. (2012), Zuberi *et al*. (2008), and Demirtunc *et al*. (2009). showed similar results. However, study by Hekimsoy *et al*. (2004) had reported contrasting findings. In the present study the mean platelet volume was lower in the diabeticcases than in the non-diabetic controls. However, multiple studies including Shah *et al*. (2004), Ates *et al*. (2009), Hekimsoy *et al*. (2004), Demirtunc *et al*. (2009), Zuberi *et al*. (2008), Jindal *et al*. (2011). Papanas *et al*. (2004), and Thomas *et al*. (Thomas, 2012) found contrasting results.In the present study the mean glycemic control of the diabetics utilized for our study was poorly controlled (149.45±67.54 mg/dl). Multiple factors like poordiet, poor lifestyle may contribute to poor glycemic control. Asignificant positive relationship between the MPV and glycemic control (fasting blood sugar) was observed in the present study . Shah *et al*, (Shah, 2012) also found that a statistically significant positive relationship. here the authors had tested values of HBA1c and fasting blood sugar levels, in another study by Thomas *et al* and Demirtunc *et al*, similar results were seen (Thomas, 2012; Demirtunc, 2009). This indicates that achieving good glycemic control may limit platelet activation, and delay the onset or progression of microvascular complications in diabetics.

However, Thomas *et al* and Hekimsoy *et al*. (Thomas, 2012; Hekimsoy, 2004) found contrasting finding in their research. This perhaps points to various other mechanisms or factors which may account for the thrombotic potential of diabetics with time (Maegdefessel, 2010; Duerschmied, 2012). Further studies on larger scale and in greater detail would be needed to correlate the relationship. In the present study there was a positive correlation that was observed between the MPV and BMI, unlike Thomas *et al* and Hekimsoy *et al*, who found no relation (Thomas, 2012; Hekimsoy, 2004).

CONCLUSION

The present study showed that the diabetics on treatment had higher mean platelet count than that of non-diabetics controls, it was also noted that mean platelet volume was lower in controls thanin cases

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