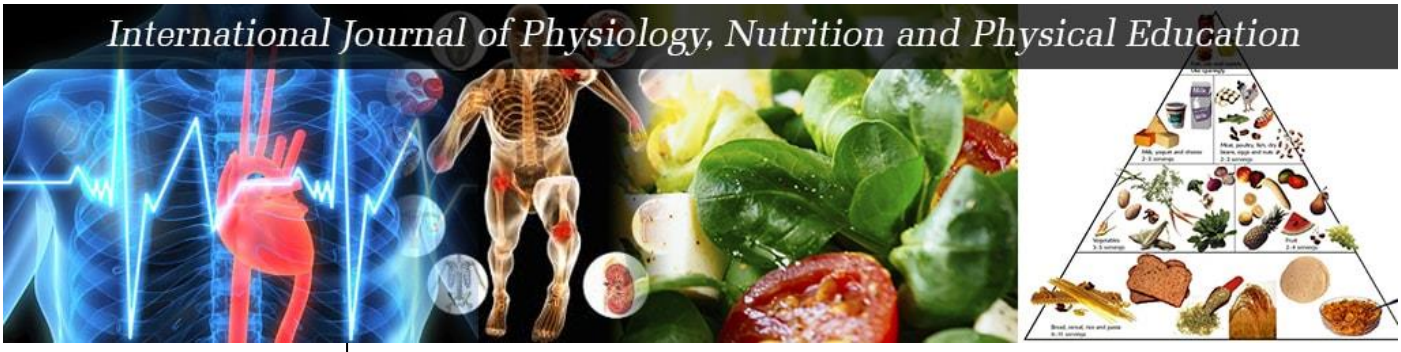

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A study of mean platelet volume in patients with diabetic and non-diabetic subjects

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Abstract
Large platelets are more thrombogenic and thus put the patient at a higher risk status. Mean platelet volume (MPV) is a determinant of platelet functionality and increased MPV is associated with increased risk for myocardial infarction, stroke and transient ischaemic attacks. The present study to estimate MPV in patients with diabetes mellitus (DM) and non-diabetic controls. Mean Platelet Volume in Diabetics and Non-Diabetics. Mean platelet volume (MPV) test was conducted in 100 diabetic patients and 100 non-diabetic subjects of age between 28-58 years, all subjects were recruited from dept of Physiology Maharajah's Institute of Medical Sciences (MIMS), Nellimarla in Vizianagaram district of Andhra Pradesh. The Mean Platelet Volume is high in Diabetics and the values are statistically significant (p value = < 0.0001). 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.

Keywords: Mean platelet volume, platelet count, ischemia, diabetes mellitus

Introduction
Diabetes mellitus (DM) is a major global health problem. According to estimates of the World Health Organization, there were 346 million people suffering from diabetes worldwide. The increased platelet activity is emphasized to play a role in the development of vascular complications of this metabolic disorder. 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. Larger platelets are more potent than smaller platelets and are hence thrombogenic. Larger and younger platelets are considered to be more reactive.
The Mean platelet volume (MPV) is the indicator for platelet function. Increase in MPV has been observed in patients with metabolic syndrome, stroke and diabetes mellitus. MPV, a determinant of platelet function, is a newly emerging risk factor for atherothrombosis. Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischaemia and transient ischaemic attacks. Platelet count and MPV are simple, effective and cheap tests that may be used to predict angiopathy in type 2 DM.
Elevated MPV has been documented to predict bad outcome for acute ischaemic cerebrovascular events independent of other clinical parameters. This study aimed to establish variations in platelet counts and mean platelet volume in type 2 diabetic patients on treatment and non-diabetic controls. To the best of our knowledge this study is novel in our environment and will serve as a foundation for other researchers in this field.

Methodology
Mean platelet volume (MPV) test was conducted in 100 diabetic patients and 100 non-diabetic subjects of age between 28-58 years, all subjects were recruited from dept of Physiology Maharajah's Institute of Medical Sciences (MIMS), Nellimarla in Vizianagaram district of Andhra Pradesh. Subjects having idiopathic thrombocytopenic purpura and iron deficiency anaemia, acute poststreptococcal glomerulonephritis, renal failure, cyanotic congenital heart diseases and myocardial infarction were excluded.

The following tests were performed to assess platelet hyper activity in diabetes mellitus:

Specimen collection: Subjects under this study were advised to fast over night (twelve hours). Blood samples were collected in fasting condition. 5ml venous blood was collected from the each subjects and it was transferred to the Plain tube and serum is separated by centrifugation and stored at -20^0 C for measured. Haemolysed and lipemic samples are avoided. For adequate quality control both normal, abnormal reference control serum solutions and calibrators were run before each testing. Other factors influencing the quality like proper functioning of instrument, glassware, cuvettes and distilled water were taken care.

Estimation Of Plasma Glucose by the Glucose Oxidase and Peroxidase (GOD POD) method using a commercially available kit Human (gmbh Germany) using Humastar 300 chemistry analyzer (Human gmbh Germany). Enzymatic colorimetric test for glucose method without deproteinisation. Glucose is determined after enzymatic oxidationn in the presence of glucose oxidase. Hydrogen peroxide formed in catalysed by peroxidase to release nascent oxygen. Oxygen is turn for reaction.

Reaction Principle: GLUCOSE + O2+ H_20 → GLUCONIC ACID + $H2O_2$

H_2O_2+Phenol + 4- Aminantipyrine $\xrightarrow[GOD]{POD}$ Red quinonimine complex + H_2O

Performance characteristics: The test is linear up to glucose conc. of 400 mg /dl or 22.2mmol/l. If the glucose concentration of sample is over this limit, sample is diluted among distilled water and the test is repeated. The result is multiplied by 3.

The MPV is the determinant of platelet functionality. MPV is a measurement of average size of platelets. MPV is Samples for platelet counts and MPV were collected using sodium citrate or ethyl diamine tetra acetate (EDTA) as anticoagulant and were done on a Sysmex auto-analyser. Well mixed blood sample was aspirated, by letting the equipment sampling probe into the blood sample and then pressing the start button. Approx. 20ul 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.

Determination of platelet count was done by using Beckman colter 5 parts differential analyzer. The blood was collected by veni puncture by using aseptic conditions and the blood was mixed with anti coagulant. The anticoagulant used here is ethyl diamine tetra acetate (EDTA) or sodium citrate.

Present study was based on Descriptive and inferential statistical analysis. Results on categorical measurements are given in Number (%) and results on continuous measurements are expressed in Mean ± SD (Min-Max) and Significance is assessed at 5% level of significance. The data took into consideration the assumptions that the Dependent variables are normally distributed, and samples drawn from the population are random, Cases of the samples are independent. The data analysis was done using the Statistical software namely, SPSS 21 version.

Results

The results of the above tests were compared between the cases (diabetics) and healthy age matched controls (Non diabetics). Values are expressed as mean ± Std.Deviation in the tables.

Table 1: Shows Various parameters Distribution in Diabetics and Non-Diabetics subjects

Status of Diabetes	Diabetics	Non-Diabetics	t-value	p-value
Age mean ± SD (years)	39.0 ± 8.25	40.12 ± 11.87	0.7748	0.4394
FBS (mg/dL) mean ± SD	155.83 ± 20.81	98.42 ± 13.74	23.0222	<0.0001
PPBS (mg/dL) mean ± SD	246.64 ± 50.36	138.63 ± 14.43	20.6179	<0.0001
Platelet count (× 103) mean ± SD	232.51 ± 18.24	212.62 ± 22.13	6.9356	<0.0001
MPV (fl) mean ± SD	9.49 ± 1.32	7.89 ± 1.02	9.5913	<0.0001

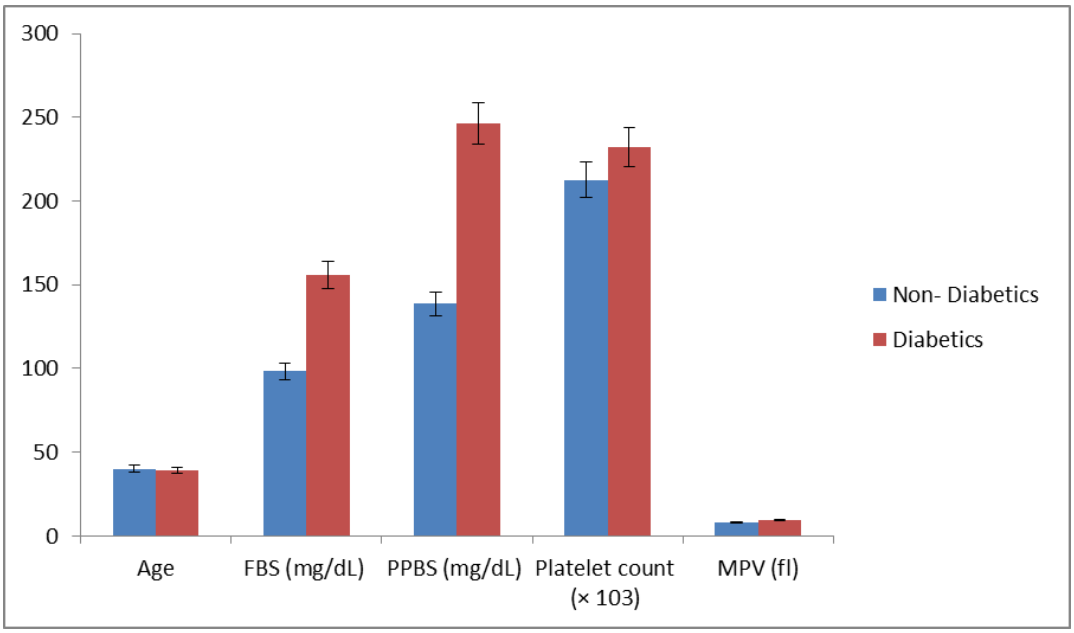

Fig 1: Comparison between Diabetics and Non-Diabetics subjects

Age distribution: In Diabetics and Non-Diabetics. The mean age for Diabetics is 39.0 and for Non-Diabetics it is 40.12. P value and statistical significance: The two-tailed P value equals 0.4394 by conventional criteria, this difference is considered to be not statistically significant. Confidence interval: The mean of Group One minus Group Two equals -1.1200 and 95% confidence interval of this difference: From -3.9706 to 1.7306. Intermediate values used in calculations: t = 0.7748, df = 198 and standard error of difference = 1.446

FBS distribution: In Diabetics and Non-Diabetics. The mean FBS for Diabetics is 155.83 and for Non-Diabetics it is 98.42. FBS values in Diabetics and Non-Diabetics. FBS values are more in Diabetics and the values are statically significant. (p value = <0.0001). PPBS distribution in Diabetics and Non-Diabetics. Confidence interval: The mean of Group One minus Group Two equals 57.4100 and 95% confidence interval of this difference: From 52.4924 to 62.3276. Intermediate values used in calculations: t = 23.0222, df = 198 and standard error of difference = 2.494.

PPBS distribution: In Diabetics and Non-Diabetics. The mean PPBS for Diabetics is 246.64 and for Non-Diabetics it is 138.63. PPBS values in Diabetics and Non-Diabetics. PPBS values are more in Diabetics and the values are statically significant. (p value = <0.0001). Confidence interval: The mean of Group One minus Group Two equals 108.0100 and 95% confidence interval of this difference: From 97.6793 to 118.3407. Intermediate values used in calculations: t = 20. 6179, df = 198 and standard error of difference = 5.239

Platelet Count Distribution: In Diabetics and Non-Diabetics. The mean Platelet Count for Diabetics is 232.51 and for Non-Diabetics it is 212.62. Platelet Count is high in Non-Diabetics and the values are statistically significant (p value =0.0001). Confidence interval: The mean of Group One minus Group Two equals 19.8900 and 95% confidence interval of this difference: From 14.2346 to 25. 5454. Intermediate values used in calculations: t = 6.9356, df = 198 and standard error of difference = 2.868.

Mean Platelet Volume distribution: In Diabetics and Non-Diabetics. The mean Mean Platelet Volume for Diabetics is 9.49 and for Non-Diabetics it is 7.89. Mean Platelet Volume in Diabetics and Non-Diabetics. The Mean Platelet Volume is high in Diabetics and the values are statistically significant (p value = < 0.0001). Confidence interval: The mean of Group One minus Group Two equals 1.6000 and 95% confidence interval of this difference: From 1.2710 to 1.9290. Intermediate values used in calculations: t = 9.5913, df = 198 and standard error of difference = 0.167.

Discussion

Diabetes Mellitus is a complex metabolic syndrome characterized by chronic hyperglycemia resulting in complications affecting the peripheral nerves, kidneys, eyes, and micro and macro vascular structures. The prevalence of all types of diagnosed diabetes in most western societies is 3-7%. Kodiatte TA, *et al*, reporting Countries with the highest absolute number of diabetics are India (19million), China (16million), and the United States (14million). The prevalence of diabetic micro vascular complications is higher in people with poor glycemic control, longer duration of diabetes mellitus, associated hypertension, and obesity. Zimmet P. *et al*. shows this leads to increased morbidities and mortalities in diabetes mellitus. Diabetes and its vascular complications can cause a financial havoc, become a burden to a country's national economy and its growth. India, having the highest number of diabetics, faces such issues. Ferroni P *et al*, reporting mean platelet volume canbe used as a simple economical test in the monitoring of DM and thereby help to decrease the morbidity and mortality. Sustained hyper glycemia leads to a series of inter related alterations that can cause evident endothelial dysfunction and vascular lesions in diabetic complications. Formation of advanced glycation end products and activation of protein kinase C are the possible mechanisms by which increased glucose induces vascular abnormalities.

Stegner D, *et al*. 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. In response to stimuli generated by the endothelium of blood vessels, platelets change shape, adhere to sub endothelial surfaces, secrete the contents of intracellular organelles, and aggregate to form a thrombus. These pro aggregatory stimuli include thrombin, collagen, epinephrine, ADP (dense storage granules), and thromboxane A2 (activated platelets). Thus, platelets may assume an important role in signaling of the development of advanced atherosclerosis in diabetes. 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. All these can produce a pro-coagulant effect and cause thrombotic vascular complications. High MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role. Thus, DM has been considered as a "prothrombotic state" with increased platelet reactivity. Platelet hyperactivity has been reported in diabetics.

Platelet hyper reactivity and increased baseline activation in patients with diabetes is multi factorial. Kakouros N, *et al*, It is associated with biochemical factors such as hyperglycemia and hyper lipidemia, insulin resistance, an inflammatory and oxidant state and also with increased expression of glycoprotein receptors and growth factors. Hyperglycemia can increase platelet reactivity by inducing non enzymatic glycation of proteins on the surface of the platelet, by the osmotic effect of glucose and activation of protein kinase C. Such glycation decreases membrane fluidity and increases the propensity of platelets to activate. Platelet function is directly regulated by insulin via a functional insulin receptor (IR) found on human platelets. *In vivo* experiments have confirmed that insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonists in healthy non obese individuals. Tousoulis D *et al* shows inflammation of superoxide increases intra platelet release of calcium after their activation, thus enhancing platelet reactivity. Furthermore, superoxide limits the biologic activity of nitric oxide (NO) because the oxidative stress impairs endothelial function that reduces production of NO and prostacyclin. Decreasing the effect of NO brings about increased platelet reactivity. Platelets from patients with diabetes express more surface P-selectin and glycoprotein (GP) IIb/IIIa receptors and are more sensitive to agonist stimulation than platelets from patients without diabetes. Platelets in DM have dysregulated signaling pathways that lead to an increased activation and aggregation in response to

a given stimulus (platelet hyper-reactivity). Platelet activation contributes to the pathology by triggering thrombus formation and causing microcapillary embolization with the release of constrictive, oxidative, and mitogenic substances such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) that accelerate progression of local vascular lesions like the neo vascularization of lens in diabetic retinopathies.

Conclusion

This study revealed a higher mean platelet count for diabetics on treatment than for non-diabetics controls while mean platelet volume was lower in cases than controls. However, both parameters in diabetics on treatment were within normal reference ranges of healthy individuals.

Conflict of interest: None declared

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