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Original Research Article

Evidence for Platelet Activation According To Some Platelet Indices in a Cohort of Type 2 Diabetic Mellitus Patients

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Abstract

Diabetes mellitus has over the years become a public health issue. It is a complex disease which is characterized by chronic hyperglycemia. The present study was designed to determine some platelet indices in Type 2 Diabetic Mellitus (T2DM) patients compared to non-diabetic controls. A total of 240 subjects comprising 120 T2DM (60 males and 60 females) aged 20-55 years and 120 apparently healthy age and gender-matched controls were recruited for the study. Blood samples (5.0ml) were collected from each subject for the analysis of the parameters using Mindray 530 BC automated analyzer, Mindray, Japan. The data was analyzed using T-test and level of significance set at $p < 0.05$. The result revealed significant increase in the platelet indices involving the Mean Platelet Volume (MPV) (12.17 $\pm$ 0.78 vs 7.80 $\pm$ 1.18), Platelet Distribution Width (PDW) (14.41 $\pm$ 1.5 vs 9.30 $\pm$ 0.92) and plateleterit (PCT) (0.27 $\pm$ 0.32 vs 0.17 $\pm$ 0.00) between the T2DM patients and non-diabetic controls. This finding supports platelet activation in T2DM patients.

Keywords: T2DM, platelet activation, platelet indices, plateletcrit, platelet distribution width, mean platelet volume.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by abnormal carbohydrate metabolism resulting in chronic hyperglycemia caused by defective insulin production, action or both [1, 2]. Type 2 Diabetes Mellitus (T2DM) is the most prevalent type of diabetes and accounts for about 90-95 diabetes cases [3-5]. Its global prevalence has increased from 4.7% (108 million) in 1980 to 9.3% (463 million) in 2019 and is postulated to increase to 10.2% (578 million) by 2030 as well as 10.9% (700 million) by 2045 [6. 7]. It is also estimated that 15.5% (9.8-27.8 million) people have type 2 diabetes mellitus with Nigeria having the highest burden of cases [8]. Platelets are small, anucleated, discoid-shaped cells that emanates from precursor cells known as megakaryocytes which are part of the hemopoietic cell line [9, 10]. They play major role in maintaining homeostasis [11]. Platelet activation has been linked with pathological processes in T2DM while a combination of platelet indices involving the plateletcrit (PCT), platelet distribution width (PDW) and mean platelet volume (MPV) has been identified as efficient marker of platelet activation [12]. Although platelet indices has been studied extensively in T2DM patients in other populations, there is currently a paucity of published data on platelet indices in T2DM patients in the Enugu State University of Science and Technology Teaching Hospital. The aim of the present study was therefore to determine the platelet indices in T2DM patients compared to non-diabetic controls.

MATERIALS AND METHODS

Study Area

The study was conducted in the Enugu State University of Science and Technology Teaching Hospital, Parklane, Enugu State, Nigeria. The State derived its name from its capital and largest city, Enugu. It has an area of 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of the South Eastern Nigeria. It lies between longitudes 6^o ^{30}'E and 6^{o}55'E and latitudes 5^o 15'N and 7^o 15^{o}E. It consists of three senatorial divisions namely Enugu East, Enugu North and Enugu West [13]. The teaching hospital is the major tertiary health facility for the State and is

located at the centre of the Enugu metropolis (Parklane) for easy accessibility to residents.

Study Design

This was a cross-sectional case-controlled survey in which patients with type 2 diabetes mellitus served as the cases while age-matched healthy non-diabetic served as the controls.

Ethical Considerations

Ethical clearance was obtained from the Ethical Review Committee of the ESUT Teaching Hospital (ESUT NP/C-MAC/RA.034/Vol. 1/290) as well as informed consent from the patients.

Sample Size

The sample size for the study was calculated using the Leslie Kish formula [14].

$$N = \frac{Z^{\alpha 2}\, PQ}{D^2}$$

Where,
n = minimum required sample size
2α = the α level of the coefficient interval or the standard normal deviate set at 1.96 corresponding to the 95% confidence interval.
P = the proportion in the target population estimated to have diabetes mellitus 8.0% [15]
D = the width of the confidence interval set at 0.05
Q = (1-p); the proportion of non-occurrence.

Substituting into the formula:

$$n = \frac{1.96 \times 1.96 \times 0.08(1-0.08)}{(0.05)^2}$$

$$= 120$$

Because the population of study is less than 10,000 the formula below was incorporated to calculate the actual sample size.

$$nf = \frac{n}{1+n/N}$$

Where,
nt = desired sample size when population is < 10,000
n = desired sample size when population is > 10,000 = 120
N = estimate of the population size = 250

Substituting:

$$nf = \frac{120}{1+120/250}$$

$$= 81.08$$

For the purpose of non-compliance which may arise on the course of subject recruitment, 10% of the nf was added, 10% of 1.08 is 8.108. Therefore, the sample size for the study is 81.08 + 8.108 = 89.188, this was approximated to a minimum size of 89 subjects.

Subject Recruitment

Subject selection was based on a simple random sampling procedure from a population of diabetic patients who gave their consent and has met the inclusion criteria.

Inclusion Criteria

1. Patients already diagnosed with Type 2 Diabetes mellitus (T2DM).
2. Non-diabetic individuals without known coronary artery disease, cerebrovascular disease, peripheral vascular disease or any systemic disease.
3. Age between 20 – 55 years.
4. Gender of both males and females

Exclusion Criteria

1. Male with hemoglobin below 13g/dl and females with hemoglobin below 12g/dl.
2. Subjects with abnormal platelet count.
3. Non-diabetics with coronary artery disease, cerebrovascular, peripheral vascular disease, systemic diseases and diabetics on antiplatelet drugs such as aspirin or clopidogrel.
4. Subjects diagnosed with any form of tumor or malignancy.

Blood Sample Collection

Blood was collected from subjects using venipuncture [16]. Subjects were made comfortable in a sitting position. A tourniquet was gently applied 2-5cm just above the antecubital fossa. The antecubital fossa was cleaned using a 70% alcohol in cotton wool. A hypodermic syringe and 21G needle was inserted into the lumen of the antecubital vein and five milliters (5ml) of blood was drawn quickly by a non traumatic pulling of the syringe piston. This was dispensed into an EDTA bottle which was gently mixed.

Determination of the Platelet Indices

Platelet indices involving the mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) were determined by performing an automated full blood count using Mindray 530 BC autoanalyzer, Japan. The sample was aspirated by letting the machine sample probe into the blood sample and then pressing the probe button. Approximately 20ul of blood was aspirated by the machine. The result of the MPV, PDW and PCT were displayed in the screen after about 30 seconds as part of the full blood count result. A printout copy of results was released on he thermal printing paper.

Data Analysis

Data was analyzed using SPSS version 23 (SPSS Inc. Chicago). Statistical significance was

defined as p<0.05. Differences in the platelet indices between the cases and controls was tested using T-test.

RESULTS

The values of the platelet indices revealed significant increase in the mean platelet volume (MPV), platelet distribution width (PDM) and the plateletcrit (PCT) in the type 2 diabetic patients (T2DM) compared to the controls (Tables 1). The Posthoc analysis confirmed significant increase in the platelet indices (MPV, PDM and PCT) between the T2DM cases and controls (Table 2) and no significant differences between the male and female cases as well as the male and female controls (Table 3).

Table 1: Mean values of platelet indices of Type 2 diabetic mellitus cases and controls

Parameters	Reference Range	T2DM (n = 120)	Controls (n = 120)	T-test (p-value)
MPV (fl)	9.0-13.0	12.17+0.78	7.80+1.18	0.001
PDW (%)	10.0-18.0	14.41+1.5	9.30+0.92	0.004
PCT (%)	022-4.4	0.27+0.32	0.17+0.01	0.036
FBS (mmol/l)	3.6-5.6	9.6+1.21	3.6+0.36	0.021
HbAIC (%)	<7	9.54+2.02	3.86+1.12	0.007

Key: MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Plateletcrit, FBS = Fasting Blood Sugar, HbAIC = Glycated hemoglobin, p<0.05* significant.

Table 2: Platelet indices of Type 2 diabetic patients and controls based on gender

Parameters	Male (test)	Female (test)	Male (control)	Female (control)	P-value
MPV	12.65 + 0.089	11.98 + 0.65	7.67 + 1.22	7.91 + 1.22	0.001
PDW	14.12 + 1.52	14.38 + 0.99	9.33 + 1.0	9.27 + 0.91	0.012
PCT	0.27 + 0.37	0.25 + 0.30	0.16 + 0.05	0.16 + 0.03	0.043

Key: MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Plateletcrit,

Table 3: Posthoc Analysis of the platelet indices of Type 2 diabetic patients and controls

Group		MPV	PDW	PCT
T2DM	Male vs T2D female	0.021	0.453	0.010
T2DM	Male vs control (male)	0.010	0.010	0.605
T2DM	Male vs control (female)	0.010	0.001	0.594
T2DM	Female vs control (male)	0.010	0.001	0.110
T2DM	Female vs control (female)	0.001	0.036	0.087
Male	Controls vs female control	0.055	0.903	0.938

Key: MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Plateletcrit,

DISCUSSION

Platelet activation leading to thrombotic state has been suggested as a major pathological process in Type 2 diabetes mellitus [17]. The combination of plateletcrit (PCT), platelet distribution width (PDW) and the mean platelet volume (MPV) have been shown to be an efficient marker of subclinical platelet activation in various diseases [18]. In the present study, we recorded significantly increased PCT, PDW and MPV in the patients with Type 2 diabetes mellitus compared to the non-diabetic controls. This is similar to the findings of Alhadas *et al*., [19] who also recorded significantly increased PCT, PDW and MPV in Type 2 diabetes mellitus patients compared to non-diabetic controls but at variance with the findings of Akinsegun *et al*., [20], Swaminathan *et al*., [21] and Tejeswini *et al*., [22] who recorded only significant increases in the MPV of T2DM patients compared to non-diabetic controls. Similar studies by Kathikeyan *et al*., [23], Shilpi and Potekar [24] and Jaben *et al*., [25] recorded significant increases in the MPV and PDW while Gowthan [26] recorded significant increase in the MPV and PCT. The MPV, PDW and PCT indirectly reflects the morphological and functional status of platelets. The MPV is a measure of platelet activity. Plateletcrit is a measure of the total platelet mass while PDW is a measure of platelet size. An increased MPV signifies increased amount of functional platelets, an increased PCT signifies a larger volume occupied by the platelets in the circulation while increased PDW reflects increased amount of large reticulated platelets in the Type 2 diabetes subjects [27]. Alterations in platelet indices in T2DM has been suggested to be as a result of chronic hyperglycemia through promotion of glycation of platelet proteins [28]. The glycation of platelet proteins may act as stimuli leading to platelet activation which manifests as increased secretion of granule contents, expression of markers facilitating adhesion and aggregation [12, 30].

CONCLUSION

The findings of the present study which revealed increased platelet indices in the Type 2 diabetes patients supports the claim for platelet activation in Type 2 diabetes mellitus and the use of antiplatelet drugs in the management of patents.

Routine monitoring of the MPV, PDW and PCT are highly recommended for Type 2 diabetes cohorts in addition to glycemic control.

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