Platelet function, anthropometric and metabolic variables in Nigerian type 2 diabetic patients

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This study examined the effects of anthropometric variables and metabolic imbalance on platelet aggregation in diabetic patients. A total of 109 volunteer were used; 58 diabetes mellitus (DM) patients (28 males and 30 females) who were receiving treatment at the University College Hospital Ibadan and 51 non diabetic control recruited from residents of Agbowo and Teachers of some secondary schools within the University of Ibadan. Body mass index (BMI) and body surface area (BSA) were assessed as indices of anthropometry, fasting blood sugar (FBS), plasma cholesterol and triglycerides (TAG) were determined using standard method and platelet aggregation test was done on the whole blood. Platelet aggregation ratio was higher in non diabetic compared to the diabetic subjects (P<0.001). The mean platelet aggregation ratio was also significantly higher in the male diabetic when compared to the female diabetic group (P<0.001). There was a significant linear relationship between platelet aggregation ratio and BMI (P<0.01), age (P<0.05), FBS (P<0.01), plasma cholesterol (P<0.01) and plasma TAG (P<0.05). However, the correlation coefficient between platelet aggregation ratio and BSA is not significant. In the non diabetic control subjects the correlation coefficient is not significant. Findings from this study suggest that, the increased platelet aggregation found in diabetic patients increased significantly with increased BMI but decrease with age. The mean platelet aggregation is also increased significantly with increase metabolic imbalance.

Key word: Platelet aggregation, anthropometry, diabetes mellitus, Body mass index (BMI), fasting blood sugar (FBS).

INTRODUCTION

Diabetes mellitus is a major degenerative disease in the world today (Ogbonnia et al., 2008). It is a serious lifelong condition that affects an estimated population of about 366 million adults, aged 20 to 79 years (IDF, 2011) and about 4.5% (14.7 million) of this population are in Africa. About 80% of this population remains undiagnosed (11.6

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Abbreviations: BMI, Body mass index; BSA, body surface area; FBS, fasting blood sugar; TAG, triglycerides.
The metabolic abnormalities that characterize diabetes such as hyperglycemia, increased free fatty acid and insulin resistance each provoke molecular mechanisms that alter platelet function and increase the production of several prothrombotic factors (Li et al., 2001; Mark et al., 2003; Asset et al., 2001). Evidence suggests that metabolic disorders frequently accompany excess body weight (Corsonello et al., 2003). Therefore, it is likely that BMI affects platelet aggregation in patients with diabetes mellitus. As far as we know no work has been done in this regard in Nigerian diabetic patients. Therefore, the aim of this study is to determine the effect of BMI, Age and BSA on platelet aggregation in African patients with diabetes mellitus.

### MATERIALS AND METHODS

#### Study design and methods

The study was carried out on patients with diabetes mellitus attending the clinic at the University College Hospital Ibadan and Staff of the University of Ibadan and Abadina College University of Ibadan, who have volunteered to participate in the study. Ethical approval was granted from UI/UCH joint ethical committee (UI/UCH ethics committee assigned number: UI/EC/09/0101). The consent of the volunteers was obtained by a signed informed consent form. The procedure involved and the rationale behind the study was explained to the subjects. A total of 58 diabetic patients attending clinic at the University College Hospital Ibadan and 51 non diabetic control subjects from staff of University of Ibadan and Abadina College University of Ibadan were compared in this study. These include 28 males and 30 females, 25 males and 26 females for both diabetic patients and control subjects, respectively. The duration of the study was 8 weeks. The subjects were instructed to fast for about 12 h prior to the beginning of the study.

#### Determination of platelet aggregation ratio

Platelet aggregation test was done on the whole blood based on the principle of the methods of Wu and Hoak (Wu and Hoak, 1974). This test is based on the principle that circulating platelet aggregates are fixed when exposed to a mixture of formalin and EDTA. The fixed platelet aggregates settle down on centrifugation, leaving a platelet rich plasma. The platelet aggregation ratio is 1 in the absence of aggregation (Ogunlade and Fasanmade, 2001). About 2 ml of blood was collected from an arm vein in the morning after overnight fast into a plastic syringe. This was gently dispensed into EDTA tube and EDTA/Formalin tube labelled according to their code. 0.1 ml of both EDTA and EDTA/Formalin sample was pipette to clean plain tubes and this was mixed with 1.9 ml of 1% ammonium oxalate, to allow it lyse the red blood cell. The samples were left on the laboratory table for 15 min at room temperature. Platelet count of samples in EDTA and EDTA/Formalin was manually determined using improved Neubauer counting chamber under light microscope. The platelets appeared under ordinary illumination as small (but not minute) highly refractive particles when viewed with the condenser racked down. The number of the platelets seen in an area of 1 mm² was counted and the number noted.

Platelet aggregation ratio was calculated as:

$$\text{Platelet aggregation ratio} = \frac{\text{Platelet count in EDTA/Formalin}}{\text{Platelet count in EDTA}}$$

Determination of body mass index (BMI) and body surface area (BSA)

Weight and height were measured without shoes using standard weigh balance and standard meter rule and BMI and BSA were determined from the formula as given below:

$$\text{BMI} = \frac{\text{Weight} \times \text{Height}^2}{\text{10000}}$$

Weight is measured in kg and height in cm.

Determination of FBS, plasma cholesterol and plasma TAG

Fasting blood sugar (FBS) was determined using one touch ultra-glucometer. The principle of which is based on the glucose oxidase method. Plasma cholesterol and plasma TAG were determined spectrophotometrically using Randox cholesterol and TAG kit (Randox laboratory UK).

### RESULTS AND DISCUSSION

The mean platelet aggregation ratio was significantly higher in the nondiabetic group (0.84±0.02) when compared with the diabetic group (0.57±0.02) (p< 0.001) (Figure 1). Also, the mean platelet aggregation ratio was significantly higher in the male diabetic (0.67±0.02) when compared with the female diabetic group (0.47±0.02) (P< 0.001) (Figure 2). In diabetic subjects there was a significant linear relationship between platelet aggregation ratio and age (P<0.05) as well as BMI.
Figure 1. Comparison of mean platelet aggregation ratio between diabetes and non diabetes. N= non diabetes 51; diabetes 58; *** = P< 0.001.

Figure 2. Comparison of mean platelet aggregation ratio between male and female diabetic patient. N= male diabetic 28; female diabetic 30; *** = P< 0.001.
(P<0.01) (Figures 6 and 7). However, there was no significant association between platelet aggregation ratio and BSA (Figure 8). The correlation coefficient in the case of age was positive (0.307) and negative in the case of BMI (-0.395). There was also a significant linear association between platelets aggregation ratio and Plasma Cholesterol (P<0.01), Plasma TAG (P< 0.05) and Fasting Blood Sugar (FBS) (P<0.01) with platelet aggregation ratio (Figure 9 to 11). In the nondiabetic control group, the correlation coefficients between age, BMI and BSA with platelet aggregation ratio were not significant (Figures 3, 4 and 5). The correlation between plasma cholesterol, plasma TAG and FBS, with platelet aggregation ratio was however not significant in the nondiabetic control group (Figure 12 to 14).

Findings obtained from this study confirm that platelet aggregation is significantly higher in diabetic compared to the non diabetic control subject. This is consistent with report from other populations and in vitro studies (Health et al., 1971; Sagel et al., 1975; Malik et al., 2012). Various mechanisms have been suggested to be responsible for this enhanced platelet activation and aggregation such as abnormal Ca²⁺-ATPase activity (Rosado et al., 2004; Jardin et al., 2006), impaired Ca²⁺ homeostasis (Ishii et al., 1991), impairment in platelet signalling such as nitric oxide (NO) production (Trovati and Anfossi, 2002). However, in vitro studies by Kutti et al. (1986) showed no increase in adenosine diphosphate (ADP) sensitivity of platelet from diabetic patients. According to Malik et al. (2012), the reason for the contrasting result could be the fact that all the patients in the study by Kutti et al. (1986) were insulin dependent diabetics. And as described by Harrison and his colleague (1980), chronic use of insulin administration may restore prostacyclin PGI2 production in platelets of diabetic animals leading to a decrease in their aggregation tendency.

Results from this study also confirm that platelet aggregation in female diabetic patients is greater than the male diabetic patients. This is line with the findings of a study done by Kueh et al. (1982) who reported an increased platelet aggregation in female diabetics over the male diabetic group. The fact that diabetes increases the incidence of myocardial infarction, claudication and stroke more in women than in men with diabetes is well established in the literature (Kannel and McGee, 1985). These cardiovascular abnormalities may be secondary to platelet aggregation.

Diabetes mellitus is associated with increased occurrence of metabolic imbalance such as hyperglycemia, increased plasma cholesterol and triacylglycerol. This study showed in agreement that there is a positive correlation between these metabolic imbalance and platelet aggregation in African diabetic patients. A previous report by Valentovic and Lubawy (1985) showed that elevated glucose in vivo alters prostaglandin generation in rat platelets. Altered prostaglandin generation increases the aggregating capacity of platelets. In contrast, Malik et al. (2012) found no correlation between FBS, TAG and Total plasma...
cholesterol in Indians with early glucose intolerance or diabetic patients. The reason for this discrepancy is not known, but it may be due to geographical location, or the sample size, since our study comprise a larger sample size.

Another finding of this study is that anthropometric data
such as Age and BMI correlate with increased platelet aggregation in diabetic patients. BMI increases in direct proportion with platelet aggregation. However, the correlation between Age and platelet aggregation is in inverse proportion. The reason for increased platelet aggregation in younger diabetic patients in this study is...
Figure 8. Scatter diagram showing correlation between platelet aggregation ratio and BSA in the diabetic subject (body surface area).

Figure 9. Scatter diagram showing correlation between platelet aggregation ratio and FBS in the diabetic subject (Fasting blood sugar).
Figure 10. Scatter diagram showing correlation between platelet aggregation ratio and plasma cholesterol in the diabetic subject.

Figure 11. Scatter diagram showing correlation between platelet aggregation ratio and plasma TAG in the diabetic subject (Triglyceride).
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$$y = -0.0009x + 0.9234$$
$$R^2 = 0.0069$$

**Figure 12.** Scatter diagram showing correlation between platelet aggregation ration and FBS in the control non diabetic subject (Fasting blood sugar).

$$y = 6E-05x + 0.8273$$
$$R^2 = 0.0007$$

**Figure 13.** Scatter diagram showing correlation between platelet aggregation ration and plasma cholesterol in the control non diabetic subject.

not clear. But it may be as a result of several factors such as increased BMI, high blood glucose, triglycerides cholesterol levels.

In conclusion, this study in an African population suggests that, the increased platelet aggregation found in diabetic patients increased significantly with increases in BMI and with decrease Age. The study also suggests that, platelet aggregation in diabetic patients is associated
with the increase metabolic imbalance in the diabetic patients. The limitation of this study is that it consider a small location in Nigeria with small sample size, therefore further study is require that consider diabetic patient from several location to establish the influence of metabolic imbalance and age on platelet aggregation in Nigeria diabetic patients.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


