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Platelet aggregation in diabetic Nigerians

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Abstract
The prevalence of macrovascular thrombosis among African diabetic patients has been shown to be lower than in Caucasians. Differences in platelet aggregation may be responsible for this observation. There has been no previous study of platelet aggregation among type-2 diabetic Nigerians. The aim of this study was to investigate platelet aggregation among diabetic Nigerian patients. Platelets from 34 diabetic patients (24 males, 10 females) and 35 control subjects were studied for aggregation in response to adenosine, ADP, and adrenaline. The intensity of the aggregation was categorized as excellent (>80%), moderate (30% - 79%), and poor (<30%). Diabetic and control subjects were of similar age, and had similar platelet counts and serum antithrombin III levels. Similarly, both diabetic patients and control subjects demonstrated lower platelet aggregation to collagen, ADP and adrenaline. However, while all control subjects exhibited spontaneous disaggregation with ADP, this was not seen in 14% of the diabetic patients (P<0.05). In diabetic Nigerians, although there is increased tendency to impaired platelet disaggregation, platelet hyper-aggregability is uncommon and this may be one of the reasons for the observed low incidence of large vessel disease, especially coronary artery disease, in our patients. (Int J Diab Metab14: 33-37, 2006)

Key words: *diabetes mellitus, platelet aggregation, large vessel disease, Nigerians*

Introduction
There are wide geographical and racial differences in the incidence of atherosclerosis and reports from Africa in the seventies [1-4] demonstrated that it was rare. Williams[5] had observed that Europeans living in Africa showed an eight-fold greater risk of pulmonary thromboembolism when compared with age- and sex-matched Africans. These differences were thought to be due to differences in the pattern of serum lipids and apolipoproteins, platelet reactivity and fibrinolytic activities. Dupuy and others[6] compared healthy Europeans and Nigerians living in Zaria, Nigeria, while Bertrand[7] and co-workers compared healthy Ivorians and Europeans living in Cote d' Ivoire. Both studies suggested that reduced incidence of thrombosis in Africans may be due to relative thrombocytopenia, rapid disaggregation of platelets after aggregation with ADP and reduced ristocetin-induced aggregation in black Africans when compared to Europeans. When flowing blood is exposed to subendothelial collagen after atherosclerotic rupture or deep arterial injury, platelets interact with collagen and adhere via adhesive glycoproteins such as fibronectin, Von-Willebrand factor, and thrombospondin. Platelets are activated to release adenosine diphosphate (ADP) and thromboxane A2 and through complex reactions in which coagulation factors and platelets are recruited and aggregated. These events have led to the *in vitro* use of collagen, ristocetin, adrenaline and ADP in testing platelet aggregation. No previous study of platelet aggregation has been carried out in Nigerian diabetic patients.

Subjects and Methods
Zaria is a small semi-urban university town surrounded by villages and, because of the central location of Ahmadu Bello University Teaching Hospital, it has served as referral center for patients with various types of diseases from Northern states of Nigeria. Type 2 diabetic patients with duration of disease varying from 3-10 years were studied. They were compared to 35 controls living in Zaria and environs. The economic and educational standing of elite controls was middle class while the non-elite controls were lower class from surrounding villages. Diets in northern Nigeria are based mainly on cereals. Patients and controls had been living in Zaria and environs for the past five years at least. All the diabetic patients were poorly controlled.

Laboratory Methods
Fasting blood sugar and lipids were determined after overnight fasting. Blood sugar was measured by a glucose oxidase method. Serum levels of cholesterol and triglycerides were determined as described in previous ly published methods.[13]

Tests of platelet aggregation
Blood (9 ml) was collected from each subject in a plastic syringe and gently dispensed into 1 ml of sodium citrate (0.11 M) in a tube, which was capped immediately. Platelet rich plasma (PRP) was prepared after centrifuging for 5 min at room temperature. Platelet poor plasma (PPP) was prepared by recentrifuging the remaining blood at 2500 to 3000 rpm for 20 min. Platelet count of PRP was adjusted to 200,000 to 300,000 per ml and used for platelet aggregation in a Bryston aggregometer (Labrintec, Montpellier, France). The aggregation in PRP was induced using adenosine diphosphate, collagen, epinephrine, and ristocetin from Bio Data Corporation, Horsham, PA 19044 USA. The final concentrations in the reaction mixture of the reagents were ADP 2.0 $x10^{-5}$M; collagen 0.19mg/ml; epinephrine 1.0 x 10^{-4}M, and ristocetin 1.5mg/ml. The intensity of the aggregation was recorded on a chart and categorized as excellent (>80%), moderate (30% - 79%), and poor (<30%).

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Table 1: Age, sex anthropometric, haematological and biochemical characteristics of diabetic patients and control subjects.

Characteristic	Diabetic patients (n 34)	Control subjects (n 35)	P value
Age (years)	42 ± 6	41 ± 7	>0.5
(Range)	37- 62	33- 58	
Male: Female ratio.	2.6: 1	2.5 : 1	>0.5
BMI (Kg/M^2)	25.2 ± 1.2	25.1 ± 0.5	
Antithrombin III level (mg%)	23.3 ± 4.1 (15.25 – 34.0)	22.9 ± 4.02(16.5 – 35.5)	>0.05
Total cholesterol (mmol/L).	6.94 ± 1.25 (1.8 – 9.1)	4.24 ± 1.24 (1.6 – 6.2)	<0.05
LDL cholesterol (mmol/L).	2.69 ± 0.94 (0.9 – 5.1)	2.58 ± 0.73 (1.0 –3.6)	>0.05
Triglyceride (mmol/L).	1.92 ± 0.46 (0.7 – 2.7)	1.18 ± 0.34 (0.7 – 1.7)	<0.05
Fasting blood glucose (mmol/L).	12.4 ± 4.6 (8.0- 22.0)	3.6 ± 0.5 (3.2 – 4.3)	<0.05
Platelet count (x 10^9/L)	142.59 ± 43.67 (50.6 – 186.27)	146.56 ± 41.32 (70.2 – 212.21)	>0.05
KCCT (seconds).	50.07 ± 13.47 (36.6 – 63.54)	40.65 ± 6.18 (28.28 – 53.0)	<0.05
Prothrombin time (seconds).	13.38 ± 2.82 (10.56 – 16.2)	12.9 ± 1.14 (10.64 – 13.0)	>0.05

Table 2: Response pattern to aggregating agents (ADP And Ristocetin) in Nigerian diabetic patients and control subjects. *

Intensity of aggregation (%)	ADP			RISTOCETIN		
	DB	ELT	NELT	DB	ELT	NELT
>80% (good)	3(8.8)	-	-	9 (26.5)	4 (20)	-
30-79% (moderate)	20 (58.8)	14 (77.8)	11 (73.3)	21 (61.8)	14 (70)	7 (46.7)
<30% (poor)	11 (32.4)	4 (22.2)	4 (26.7)	4 (11.7)	2 (10)	8(53.3)
Total.	34 (100)	18 (100)	15 (100)	34(100)	20(100)	15 (100)

**Number of individuals with a given intensity of platelet aggregation is shown outside the brackets with the corresponding percentages in the brackets.*
DB = Diabetic patients.
ELT = Elite controls.
NELT = Non elite controls.

Statistical Analysis
Student's t test was used to compare differences between the means. Chi-square test was used to study differences between groups. The level of significance in each case was $p<0.05$.

Results
Table 1 shows the age, sex, anthropometric, haematological and biochemical characteristics of the diabetic patients and the controls. Complications observed in diabetic patients were peripheral neuropathy 12 (35.3%), hypertension 13 (38.2%), retinopathy 12 (35.3%),impotence 4 (11.8%), cataract 2 (5.9%), nocturnal diarrhoea 2 (5.9%), and nephropathy 3 (8.8%) athropathy 2 (5.9%),), amputation for diabetic foot 2 (5.9%), and hypertensive stroke 1 (2.94%).

Mean levels of total cholesterol, triglyceride and fasting blood glucose levels were higher in diabetics than controls ($P <0.05$). There was no significant difference between levels of anti-thrombin III in diabetics and controls. KCCT was more prolonged in diabetics than controls ($P <0.01$). Tables 2 and 3 show the intensity of reaction of platelets to the aggregating agents in diabetic patients and controls. Figure 1 depicts the tracing patterns of the reactivity to ristocetin of platelets from diabetic patients. Three (8.8%) diabetic patients had aggregation to ADP more than 80% while none of the elite and non-elite controls reacted in this range (Table 2). Other diabetic patients and elite and non-elite controls were in the moderate and poor ranges. Two (32.4%) diabetic patients were in the poor range which is significantly more than combined elite and non-elite controls ($p< 0.05$). All elite and non-elite platelets showed spontaneous desegregation while all diabetic patients except five (14.7%) exhibited spontaneous disaggregation.

Ristocetin Aggregation
Nine diabetic patients (26.5%) had aggregation above 80% as compared to 4 (20%) elite controls and none in the non-elite group. Eight of the non-elite group (53.3%) had poor reaction to ristocetin and this is more than in the elite (2 or 10%) and diabetic (4 or 11.7%) groups. The members of the elite group are more urbanized than non-elites who are rural. The difference between ristocetin reactivity in diabetic patients and non-elite control subjects was significant ($p<0.05$) (Table 2).

Collagen aggregation
Thirty-two (97.0%) of diabetic patients, and 31 (96.9%) controls were in poor to moderate response groups ($p<0.05$). Apart from one diabetic patient and one elite control with response in the 80% and above range, all controls and

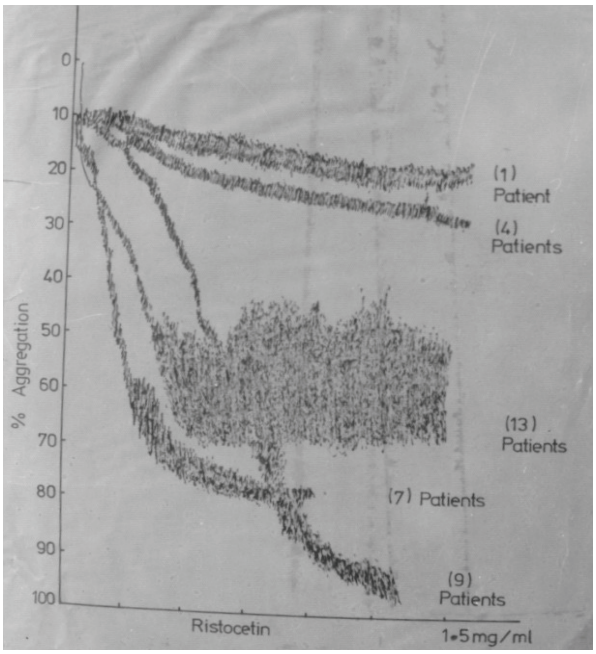

Figure 1: Platelet aggregation among diabetic patients and control subjects.

diabetic patients were in the moderate and poor responder group. A lag phase before aggregation was exhibited by all the patients and controls (Table 3).

Epinephrine aggregation

Apart from two (4.3%) from the non-elite in the 80% responder group, all the controls and diabetic patients were in the low and moderate responder groups. The difference between diabetic patients and non-elite control subjects was significant ($p<0.05$) (Table 3).

Discussion

Vascular disease, especially large vessel disease, has emerged as a major burden of diabetes leading to acute devastating events like myocardial infarction and stroke particularly in Western countries. Diabetes mellitus enhances cardiovascular risk, which is increased two to three-fold in Caucasian men and postmenopausal women and five-fold in premenopausal women.[8, 9] Abnormalities which include increased platelet adhesiveness and aggregation, endothelial cell dysfunction, decreased serum antithrombin III, increased serum concentration of von Willebrand factor and factor VIII related antigen, as well as decreased plasminogen activator, prostacyclin and plasminogen[10,-12] especially in the presence of diabetic microangiopathy, are reported in Caucasians. Furthermore, severe lipid abnormalities, which are atherogenic, are reported in Caucasians, whereas such severe lipid abnormalities in African diabetic subjects are exceptions rather than the rule.[13] Among patients with type 2 diabetes mellitus, microalbuminuria or proteinuria further doubles the cardiovascular risk.[14]

Racial and geographical factors seem to be important in the reactivity of platelets to aggregating agents. Caen[1] reported that the platelets of the inhabitants of the Andes plateau were found not to respond to aggregating agents. Williams in 1924[5] had shown that Europeans living in Africa showed an eight-fold greater risk of pulmonary thromboembolism when compared with age- and sex-matched Africans. Dupuy *et al*[6] showed that Nigerians had low ristocetin-induced reactivity (more marked in rural Nigerians) when compared to European whites and that platelet reactivity to ADP was followed by immediate and rapid disaggregation. It was further shown that the plasma of Nigerians inhibited aggregation of European platelets. Buchanan had demonstrated this tendency in American blacks when compared with American whites,[2] but the low ristocetin induced activity was not as marked as the findings of Dupuy and co-workers, neither was rapid disaggregation by ADP found. Betrand and co-workers[7] confirmed this low reactivity of platelets to ADP, collagen and ristocetin in the platelets of Ivorian blacks when compared to white Europeans living in Ivory Coast. They attributed this difference to greater alcohol consumption and more smoking among Europeans on the one hand, and more fish consumption among Ivorians on the other. Fish contains 3-polyunsaturated fatty acids that favour the production of prostacyclin, a prostaglandin known to reduce platelet aggregation. Our results have confirmed this low reactivity which is greater in the non-elite controls who are not fish eaters like Ivorians.

Table 3 : Response pattern to aggregating agents (collagen and adrenalin) in Nigerian diabetic patients and controls*.

Intensity of aggregation (%).	COLLAGEN			ADRENALINE		
	DB	ELT	NELT	DB	ELT	NELT
>80% (good)	1 (3.0)	1 (5.6)				2 (4.3)
30-79% (moderate)	7 (21.2)	8 (44.4)	6 (42.9)	6 (18.2)	8 (40)	8 (57.1)
<30% (poor).	25 (75.8)	9 (50)	8 (57.1)	27 (81.8)	12 (60)	4 (28.6)
Total.	33 (100)	18 (100)	14 (100)	33 (100)	20 (100)	14 (100)

**Number of individuals with a given intensity of platelet aggregation is shown outside the brackets with the corresponding percentages in the brackets.*

DB = Diabetic patients.
ELT = Elite controls.
NELT = Non elite controls

There is a clear difference in pattern of large vessel disease presentation in Caucasians and Africans with regard to atherosclerotic processes leading to coronary artery disease and stroke (which are the major cause of mortality in Caucasian diabetic patients). In a classic post mortem study of Nigerians with diabetes,[15] atherosclerotic plaques were found in large vessels (aorta, cerebral, carotid, femoral) in many diabetic patients with more then 10 years duration of diabetes and yet only two cases had thrombosis. In a ten-year clinical review of 1127 cases in Zaria, we found only one case of thrombosis, associated with marked hypercholesterolaemia and intimal calcification involving the abdominal aorta, femoral and iliac arteries.[16] Although a few cases of coronary artery disease have been reported in 1977[4] and recently in two elite diabetic patients in Zaria, the general trend is that coronary artery disease and large vessel peripheral artery disease is much less of a burden than problems of diabetic foot, nephropathy and neuropathy in which microangiopathy is more important. Some important protective mechanisms and factors mitigating against clinical manifestation of large vessel disease in Nigerian diabetic patients would therefore involve normal anti-thrombin III levels and low reactivity to most platelet activators as was found in this study. A rural non-elite lifestyle may promote these factors.

It is worthwhile to note that nine diabetic patients had very intense reactions (>80%) to ristocetin suggesting that uncontrolled diabetes mellitus is a risk factor for the development of excessive platelet reactivity and loss of protection to thrombosis, especially as these patients did not show spontaneous disaggregation to ADP. The tendency to higher reactivity to ristocetin in elite controls (4 or 20%) and diabetics (9 or 26.5%) as compared to non-elite controls, points to a possible enhancing role of modernization and urbanization. Although triglycerides and total cholesterol levels were higher in diabetic patients than in controls, they did not approach levels associated with clinically manifested atherosclerosis and medial calcification reported in an earlier study in Zaria.[16]

Studies[17-19] on the use of anti-platelet agents, especially low dose aspirin, in Caucasian patients demonstrated significant risk reduction for myocardial infarction and cerebral infarction although the risk of cerebral haemorrhage was increased. Recommendations of low dose aspirin have therefore been made for categories of Caucasian and Indo-Asian diabetic patients.[20-22] Our present finding may, however, create doubts as to the benefits of such intervention in Nigerian and possibly other African diabetic patients, especially those with rural lifestyles. Platelet aggregation studies should be extended to other parts of Africa and Asia[23,24] in order to obtain consensus or to observe regional or geographic differences.

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