PLATELET NUMBER AND FUNCTIONS IN DIABETIC PATIENTS IN ENUGU, NIGERIA

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Summary

Objectives: Diabetes mellitus initiates many biochemical and haematological changes in the patients. Many of these changes have been established among Caucasians with little work among blacks. We investigated the effects of this condition on some haematological parameters among blacks in an Eastern State of Nigeria.
Method: Platelet count and functions were assessed in 58 diabetics attending Diabetic Clinic of University of Nigeria Teaching Hospital, Enugu, Nigeria. Another 50 age- and sex-matched non-diabetics were used as controls. The parameters measured included fasting plasma glucose, platelet count, platelet adhesion and platelet factor 3 availability. Plasma glucose was estimated by glucose oxidase method, platelet count was done manually using ammonium oxalate method, platelet adhesion was assessed by Hellen's method while platelet factor 3 availability was assessed by Kaolin method.
Results: Our results showed no significant changes in platelet number ($p>0.05$) but significant increase in platelet adhesion ($p<0.05$) and significant decrease in platelet factor 3 availability ($p<0.05$) in diabetics. When subjects were grouped according to age and sex, the results maintained the same features. We are of the opinion that though platelet number may not change significantly in diabetes, their functions are altered. This calls for constant monitoring of these patients in course of their treatment.

Key words: Diabetes; Platelet number; Platelet function

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Introduction

Diabetes mellitus (DM) is a disorder of the endocrine function of the pancreas. It is better regarded as a syndrome, of which there are many causes (1), principally by one of two things – an insufficient production of insulin in the pancreas or the resistance of the body cells to the actions of insulin despite normal or high level of insulin. Both causes can co-exist. Experience, particularly from developed countries, indicates that with better living conditions, and increase in life expectancy, there is now an increased incidence of non-communicable diseases including diabetes (2). These diseases exert a terrible burden on health services and resources due to their chronicity and many complications. The prevalence of these diseases is rapidly growing in developing countries. This is in addition to their economic, social and other health problems like HIV/AIDS, Tuberculosis, Malaria and Malnutrition. It is expected that within the next 15 years, the incidence may be more than double of the current figure (3). There are many recorded biochemical and hematological disturbances in diabetes including hyperlipidaemia (4), ketoacidosis (5) ureamia, elevated serum transaminase (6), microalbuminuria (7), anemia and decreased fibrinolytic potential (8) among others.

Platelets, which are small enucleate discoid cell fragments shed from megakaryocytes in the bone marrow (9) have life span of 7-10 days. They are involved in haemostasis through initiation and formation of haemostatic plug to block the holes in blood vessel walls, thus leading to the repair of vascular injures. Activation of platelets is by thromboxane A_2. Inflammation and haemostasis are closely linked and are often activated concomitantly (10). When there is inflammation, leucocytes in circulation will roll and rest on endothelial cells and finally migrate into the tissue. In response to vascular damage, circulating platelets adhere to this subendothelial tissues. This recruits more platelets into aggregate that function as pro-coagulant surface (11). The above processes are linked and mediated by cells adhesion molecules including selectin, integrin, leucine-rich glycoprotein and receptors of Ig type (12). Selectin, which mediates the interactions between platelets, leucocytes and endothelial cells also stabilizes initial platelet aggregation (13) and provides signals for monocytes adhesion. It is found in the plasma, depending on time and method of sample collection and it is known to be related to platelet count. CD40 ligands, which are a member of tissue necrotic factor-alpha family are also expressed by platelets and they account for over 95% of the ligands found in whole blood (14). The soluble form of this ligand also contributes to stabilization of platelet-rich thrombi and inhibition of re-endothelization of injured vessels. Experimental studies (15) have shown that heterotypic aggregation is more dependent on platelet activation than leucocytes activation.

Platelet count in apparently healthy individuals is about $150 – 400 \times 10^9$/l. This tends to be slightly higher in women than in men, and lower in healthy West Indians and Africans than in Caucasians (16). Disorders of platelets may result from either insufficient number or inappropriate function. It is caused by either congenital diseases or acquired conditions especially in response to endocrine condition, shock, surgical procedures and menstruation. DM, which is an endocrine disorder, is considered to be a prothrombotic state with elevated platelet activation, activation of coagulation system and decreased fribrinolytic potential (8). The main factors that account for abnormal platelet function in DM include synthesis of immature, larger and more reactive platelets in the bone marrow, activation of platelets when exposed to the metabolic milieu found in DM and activation of platelets due to vascular damages. These biochemical changes that bring about these platelet dysfunctions in DM have been well documented (17, 18, 19, 20, 21, 22, 23). Incidentally all these studies have been based on the values obtained from Caucasians.

Since there is racial variations in platelet number and, probably, functions (16), there is need to study the state of platelets in African diabetics. We present this study as part of the investigations of the activities and nature of platelets in African diabetics. Ethical clearance was obtained from the Ethical Committee of University of Nigeria Teaching Hospital, Enugu, Nigeria while additional consent was sought and obtained from the subjects.

Materials and Methods

Subjects: A total of 108 subjects aged between 18 and 60 years were used for this study. 58 of them were diabetics attending Diabetic Clinic of University of Nigeria Teaching Hospital, Enugu, Nigeria. Thirty two (32) of them were males while twenty-six (26) were females. The remaining 50 subjects were age and sex-matched non-diabetics selected from students and workers of the same institution whose consents were sought. They comprised of 25 males and 25 females and formed the control group (non-diabetics).

Exclusion criteria: All the subjects (patients and control groups) were interviewed to make sure they were not having any other problems that may affect platelet number and functions. Females who were in their menstrual periods were excluded while subjects with hypertension and any other complication of diabetes were excluded. Also, only diabetics who have not taken any anti-diabetic drug for at least two weeks were considered.

Sample Collection: A total of 5.5ml of fasting whole blood was carefully collected by clean venepuncture from each subject. About 4.5ml of the blood was put into a clean glass test tube containing 0.5ml of trisodium citrate anticoagulant (32g/l) and mixed thoroughly but gently. Out of this mixture, 2.0ml was transferred to another clean test tube for platelet count and platelet adhesion test while the remaining 3.0ml was centrifuged for platelet factor 3 availability. The centrifugation was 150g for 5 minutes to obtain platelet-rich plasma and 1500g for 5 minutes to obtain platelet-poor plasma. The extra 1.0ml of whole blood was put into sodium fluoride/potassium oxalate anticoagulant container and centrifuged to obtain plasma for plasma glucose.

Methods: Plasma glucose was estimated by glucose oxidase method as earlier reported (24). Platelet count was done manually using 1% aqueous ammonium oxalate diluent as described by Bates in 2002 (25). Platelet adhesion test was done using Hellen's method as reported by Dacie and Lewis (26) while platelet factor 3 availability was assessed by recalcification of platelet rich plasma also as reported by Dacie and Lewis (26).

Results

Table 1 shows the mean values (±SD) of all the parameters measured in both patients and control subjects. The platelet count showed no significant difference ($p>0.05$) between the diabetics and non-diabetes. However, there was significant increase ($p<0.05$) in platelet adhesion and significant decrease ($p<0.05$) in platelet factor 3 availability in diabetics than in non-diabetics. Table 2 shows the results of these parameters when patients were grouped into age groups. The values showed the same trend as in table 1, signifying that age does not influence the outcome of the results both in patients and control subjects. However, when the subjects were grouped into different sexes, only the platelet count showed significantly higher values ($p<0.05$) in males than in females. This occurred in both diabetics and non-diabetics. Other parameters showed no significant difference ($p>0.05$) both in diabetics and non-diabetics (Table 3)

Table 1: Mean values (±SD) of plasma glucose, platelet count, platelet adhesion and platelet factor 3 in diabetics and non-diabetics

Parameters(units)	**Patient**	**Control**	**P-value**
Platelet count ($x10^9$/l)	255.2(69.6)	262.4(66.5)	>0.05
Platelet adhesion (%)	37.4(6.8)	32.4(6.0)	<0.05
Platelet factor 3 availability(sec)	33.5(2.0)	25.9(1.2)	<0.05
Plasma glucose (mmol/l)	18.6(4.2)	4.8(1.8)	<0.01

Table 2: Platelet count, platelet adhesion and platelet factor 3 in both diabetics and non-diabetics in different age groups.

Parameters	**Age**	**Patient**	**Control**	**P-value**
Platelet count ($x10^9$/l)	18 - 39	258.2(61.6)	262.4(66.5)	>0.05
	40 – 60	261.2(75.9)	263.1(67.5)	>0.05
Platelet Adhesion (%)	18 - 39	36.4(8.1)	32.4(6.0)	<0.05
	40 - 60	38.1(5.6)	34.1(6.4)	<0.05
Platelet factor 3 (sec)	18 - 39	33.5(1.8)	35.9(1.2)	<0.05
	40 - 60	33.5(2.1)	37.2(1.5)	<0.05

Table 3: Platelet count, platelet adhesion and platelet factor 3 in male and female diabetics and non-diabetics.

Parameter	**Subject**	**Male**	**Female**	**P-value**
Platelet count ($x10^9$/l)	Diabetics	263.4(74.9)	247.6(64.3)	<0.05
	Control	272.5(69.0)	252.2(66.0)	<0.05
Platelet Adhesion (%)	Diabetics	39.5(5.8)	35.2(7.1)	>0.05
	Control	33.1(7.5)	31.7(4.2)	>0.05
Platelet factor 3 (sec)	Diabetics	33.2(1.9)	33.8(2.0)	>0.05
	Control	35.6(0.9)	36.2(1.5)	>0.05

Discussion

DM is known to cause many biochemical sequelae. These lead to subsequent changes in some organ functions especially the kidney and heart. In some severe hyperglycaemic state, there may be reduced blood circulation, causing hypertonic plasma as well as acid-base and electrolyte inbalance. Haematologically, there may be haemoconcentration, causing increased haemoglobin concentration without increased erythropoiesis, or anemia due to kidney dysfunction or nutritional deficiency. DM is associated with accelerated atherothrombotic disease and has been demonstrated as a pro-thrombotic state with elevated platelet function (8). However, from this work, we have demonstrated that diabetes may not actually cause increase in platelet number, at least in our environment. This is seen from non-significant difference in the platelet count between the diabetics and non-diabetics. This, probably, goes to confirm that diabetes has little or no effect on the platelet-producing organ – the borne marrow. Instead, diabetes influences the platelet functions by way of activation. This is deduced from the significant increase ($p<0.05$) in platelet adhesion in diabetics followed by significant decrease ($p<0.05$) in platelet factor 3 availability. Increase in platelet adhesion could be as a result of elevated activation and other anomalies in the mechanism of action of platelets seen in diabetes mellitus. It has been noted that altered arachidonic acid metabolism in diabetes which results in increased thromboxane (potent platelet activation substance) synthesis also accounts for enhanced activation of platelets in DM (22). Also adduced to this, is the observation that platelets in diabetics show increased glyco-protein receptor binding of adhesive proteins (3). In addition, membrane fluidity is known to help in modulating cell functions by altering the receptor availability. This fluidity is reduced in diabetic platelets and therefore may contribute to hyperactivity of platelets in diabetes (27). Increased recalcification time depicted by reduced platelet factor 3 availability in diabetes is demonstrated by this work. This may possibly result from the calcium imbalance as well as dysfunctional platelets in diabetes.

A striking observation in this study is that platelet count was relatively higher in males than in females. This occurred in both diabetics and non-diabetics. This finding is in disagreement with earlier study (16), which found platelet number in females higher than in males. This difference may be caused by differences in socio-economic and nutritional status of the populations studied. It will be necessary to explore this line to establish the reference values and sex differences in platelet counts within each locality. It is also necessary to assess whether these changes in platelet functions correlate with the level of plasma glucose which may help in prognosis. Finally, it will be necessary to establish the effects of different anti-diabetic drugs, if any, on platelet number and functions and whether the duration of treatment correlates (positively or negatively) with these parameters. This will go a long way in helping physicians in their choice of drug, as well as their advice to patients when teaching them on how to treat themselves at home.

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