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Abnormalities in thrombogenic and rheologic factors in Nigerian hypertensive patients

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Summary
Platelet aggregation, plasma viscosity and plasma fibrinogen concentration were measured in 20 hypertensive Nigerian patients (10 males and 10 females) aged 32-72 years. Another 20 patients gender-matched normotensive subjects, served as controls. Platelet aggregate ratio was significantly lower ($P < 0.05$) in hypertensive (0.38±0.24) than in normotensive subjects (0.80±0.19). The relative plasma viscosity of the hypertensive patients (2.04 ± 0.14) was higher ($P < 0.05$) than the value in control subjects (1.64 ± 0.25). Plasma fibrinogen concentration, determined by the direct clot weight technique, was also significantly higher ($p<0.05$) in hypertensive patients (3.90 ± 0.62mg/dL) than in normotensive patients (2.70 ± 0.60mg/dL). There was no evidence of gender differences in all the above variables except in the plasma fibrinogen concentration, which was significantly higher ($P < 0.01$) in male hypertensive patients. It is thus evident that haemorheological and thrombogenic abnormalities may be present in hypertensive Nigerian patients at the time of diagnosis and therapeutic interventions that reduce the risk of thrombogenesis and rheologic abnormalities should be considered in the management of Nigerian patients with hypertension.

Keywords: *Platelet aggregation, plasma fibrinogen concentration, plasma viscosity, hypertension, Nigerian patients*

Résumé
La masse de la plaquette, la viscosité de plasma et la concentration fibrinogène de plasma ont été mesurées dans 20 malades hypertensifs nigérians (10 hommes et 10 femmes) agé de 32 à 72 ans. 20 autres malades dont le genre était conforme à ceux des personnes normotensif ont servi comme le groupe de contrôle. La proportion de l'ensamble de plasma était considérablement plus bas ($p<0,05$) dans les sujets hypertensifs (0,38 ± 0,24) que dans le cas de ceux qui était normotensifs (0,80 ± 0,19). La viscosité rélative de plasma des malades hypertensifs (2,04 + 0.14) était plus élevé ($p<0.05$) que la valeur des sujets du groupe de contrôle (1,64 ± 0,25). La concentration de plasma fribrinogène déterminé par la technique directe du poids de caillot était aussi considérablement plus élevé ($p<0,05$) dans les malades hypertensifs (3,90 ± 0,62mg/dL) que dans le cas des malades normotensifs. (2,70 ± 0,60 mg/dL). Il n'y avait aucune évidence de différences de genre dans les variables ci-dessus sauf dans la concentration fibronogène de plasma qui était beaucoup plus élevé($p<0,01$) chez les hommes souffrant de l'hypertension. Il est donc évident que les anormalités haemorheologique et thrombogenique peut se trouver dans les malades hypertensifs Nigérians au moment du diagnostique et de l'intervention thérapeutique et que la réduction du risque de thrombogenèse et les anormalités rheologique devraient être considérées dans la gestion de malades hypertensifs nigérians.

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Introduction

The major complications of hypertension are mainly due to thrombotic events, which result in heart attacks and strokes [1]. The presence of haemorheologic abnormalities and excessive platelet activity is a pointer to the imminent development of these complications in hypertensive patients. The incidence of these complications becomes markedly reduced, however, with the institution of appropriate and adequate blood pressure control measures [2]. It has been suggested that there is a direct correlation between blood pressure and blood viscosity in normal and hypertensive subjects [3], suggesting that a rise in blood viscosity may be a contributing factor in the development and maintenance of high blood pressure in hypertensive patients. The rise in fibrinogen level in hypertensive patients [4,5] may be a significant contributing factor for the increased plasma viscosity in these patients.

Patients with hypertension also tend to have a high prevalence of atherothrombotic accidents, with considerable evidence that platelets also play an important role in the development and progression of atherosclerosis in these patients [6]. Platelet aggregation is a prelude to intravascular coagulation as the aggregated platelets provide the nidus for fibrin deposition. Platelet aggregation has been variously reported to be normal [7,8] or abnormal [9,10] in uncomplicated hypertension. It is known that all platelet hyperactivity parameters are reduced significantly with the normalization of blood pressure [11]. Although platelet aggregation studies have not been carried out in Nigerian hypertensive patients to the best of our knowledge, atherothrombotic accidents are commonly seen as complications in those with poor blood pressure control.

The aim of this study was, therefore, to examine the status of platelet activity and plasma viscosity in Nigerian patients with recent onset of hypertension, who are without complications. This is to remove any effect of the complications and the management of hypertension may have on platelet activity and plasma viscosity. It is hoped that the results of the study will be useful in the recommendation of appropriate therapy, which will be targeted at prevention of atherothrombotic complications, as early as possible after the diagnosis of hypertension in Nigerian patients.

Subject and methods

Twenty newly diagnosed hypertensive patients (10 males and 10 females), aged 32-72 years, were recruited for this study at the medical clinics of the University College Hospital, Ibadan, Nigeria, after satisfying a set of selection criteria for the study. The first 10 patients of either sex, seen in the clinic, who satisfied the selection criteria, were included in the study. The selection criteria include the absence of diabetes, no previous antihypertensive therapy, absence of medication, which may affect platelet activity and morphology in the previous one month, absence of apparent evidence of complications of hypertension and absence of anaemia and liver disease. To be considered definitely hypertensive, a diastolic blood pressure of 100mmHg

and above must have been recorded on at least three occasions within one week. Most of the patients were referred to the medical clinics, from ophthalmology, otorhinolaryngology and surgical clinics, for the management of hypertension, which was a chance finding during surgical consultations. The twenty subjects, who served as controls, were randomly selected from hospital staff and their relatives, with the application of the same selection criteria, except that none of them was hypertensive at the time of recruitment for the study.

Blood was collected into a clean plastic syringe by venepuncture with minimum venous constriction. Plasma was prepared from part of the blood, which was added to commercially prepared specimen tubes containing ethylene di-amine tetra acetic acid (EDTA), by centrifuging at 150g for 10 minutes at laboratory temperature of 22-24°C.

Plasma viscosity (expressed as Relative Viscosity, RV) was determined by the simple syringe viscometry method of Reid and Memeh [12].

Plasma fibrinogen concentration was estimated by the direct clot weight technique [13].

Venous blood, 0.5mL, was added into each of two plastic containers, one containing 2mL buffered EDTA solution and the other, 2mL buffered EDTA/formalin solution. They were left on the laboratory table for 15 minutes at a temperature of 22-24°C after thorough mixing. They were thereafter centrifuged at 150g for 8min to obtain platelet-rich plasma (PRP) according to the methods of Wu and Hoak [14]. Platelet count of PRP samples was determined using the improved Neubauer counting chamber under phase contrast. Platelet aggregate ratio was calculated as:

$$\frac{\text{Platelet counts in EDTA formalin PRP}}{\text{Platelet counts in EDTA PRP}}$$

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 aggregate ratio is 1 in the absence of aggregation and it is thus inversely proportional to extent of platelet aggregation.

Comparisons were made in the values of the measured variables between the patients with hypertension and the normotensive controls by subjecting the data to statistical analysis, using Student t-test. The results are presented as means ± S.D. and differences in the values were considered significant at p values of less than 0.05.

Results

Table 1 shows that the mean arterial blood pressure (MAP), which is calculated as the sum of diastolic blood pressure and a third of the pulse pressure, was significantly higher ($P < 0.001$) in hypertensive patients, being 131.8 ± 7.7mmHg, than the value of 96.0 ± 6.1mmHg in control subjects. Female hypertensive patients had higher MAP than that of the males (Table 2). The difference was, however, not statistically significant ($P > 0.05$).

The relative plasma viscosity was significantly higher in hypertensive (2.04 ± 0.14) than in normotensive (1.64 ± 0.25) subjects ($P < 0.05$) as shown in Table 1, but there was no significant gender difference ($P > 0.05$) in the values (Table 2).

The plasma fibrinogen level was also significantly higher ($P<0.05$) in hypertensive (3.90 ± 0.62 mg/dL) than in the normotensive subjects (2.70 ± 0.60 mg/dL).

Table 1: Thrombogenic and rheologic indices of the hyper tensive and normotensive subjects.

	Hypertensive (n = 20)	Normosentive (n = 20)	P value
MAP (mmHg)	131.8±7.7	96.0± 6.1	<0.0001
Relative plasma viscosi	2.04± 0.14	1.64± 0.25	<0 05
Plasma fibrinogen (mg/dl)	3.90±0.62	2.70± 0.60	<0.05
Platelet aggregate ratio	0.38 ±0.24	0.80± 0.19	<0.05

Values are means ± S.D.
MAP = Mean arterial blood pressure.

Table 2: Effect of gender on MAP, relative plasma viscosity, plasma fibrinogen and platelet aggregate ratio in hypertensive and normo tensive subjects.

	Males (n = 10)	Females (n = 10)	p value
MAP_a (mmHg)	129.4 ± 8.2	134.6 ± 7.1	>0.0
MAP_b (mmHg)	95.5 ± 4.4	92.5 ± 5.3	>0.0
Relative plasma $viscosity_a$	2.08 ± 0.24	2.02 ± 0.21	>0.0
Relative plasma $viscosity_b$	1.67 ±0.41	1.61 ± 0.32	>0.0
Plasma $fibrinogen_a$ (mg/dL)	4.50 ± 0.63	3.62 ± 0.51	<0.0
Plasma $fibrinogen_b$ (mg.dL)	2.67 ± 0.7	2.68 ± 0.94	>0.0
Platelet aggregate $ratio_a$	0.39 ± 0.28	0.38 ± 0.24	>0.0
Platelet aggregate $ratio_b$	0.83 ± 0.23	0.79 ± 0.1	>0.0

Values are means S.D.
MAP = Mean arterial blood pressure.
a = hypertensive patients.
b = normotensive subjects.

As shown in table 2, hypertensive males had signifi cantly higher ($P < 0.05$) plasma fibrinogen concentration (4.5 ± 0.63 mg/dL) than hypertensive females (3.62 ± 0.51mg/dL) Platelet aggregate ratio in hypertensive patients (0.38 + /-0.24 was significantly lower ($P < 0.05$) than in normotensive subjects (0.80 ± 0.19) (Table 1). There was no significant differenc ($p > 0.05$) in the platelet aggregate ratio due to gender differenc (table 2).

MAP, relative plasma viscosity and platelet aggregat ratio were similar in men and women with normal blood pres sure.

Discussion

There was no way to ascertain the duration of hypertension i the patients used for this study. It could safely be regarded as c short duration even though the absence of complications is nc enough evidence that the hypertension, which was newly diag nosed, was of recent onset. None of the patients had previousl taken antihypertensive and antiplatelet therapy and so the va ues of the atherothrombotic and rheologic indices (platelet ag gregation, fibrinogen levels and plasma viscosity) estimated i this study could not have been as a result of the use of antihy pertensive and antiplatelet drugs.

The prevalence of hypertension in Nigeria has bee estimated, in a countrywide survey, to be 11.2% in those age 15 years and above [15]. It is lower in women than in men. I another survey of an urban population in this country, onl 19.6% of the hypertensive population knew they had hyper tension [16]. Thromboembolic phenomena are common compli cations of hypertension and hypertension is regarded as th most common cause of heart failure in Nigeria and other blac African countries [17].

The basis for the observed increase in plasma fibrinogen concentration in hypertension is not clear. However, it is known that plasma fibrinogen concentration is an important factor determining the increase in blood and plasma viscosity in hypertension [3]. The increase in fibrinogen concentration in the patients with hypertension agrees with similar observations made by previous workers [1,4]. Vaya *et al.* [18] also observed a significant increase in the fibrinogen level in male and female normotensive offsprings of hypertensive individuals. This suggest a possible genetic factor may be involved. It is apparent from previous reports that hypertensives develop vascular structural adaptations quite early and frequently present with serious arteriosclerotic vascular alterations, especially in the resistance vessels, which become less distensible than those of normotensive subjects [19,20]. Ritche and Fuller [21]. had earlier suggested that a possible pathophysiologic pathway could be that local production of fibrinogen degradation products (predominantly fragment D) is involved. This stimulates macrophages, which in turn produce the regulatory protein, hepatocyte-stimulating factor, leading to increased hepatic synthesis of fibrinogen and other acute phase proteins. It was observed that there was a gender difference in the level of plasma fibrinogen in the hypertensive patients, being higher in men than in women. This is in agreement with previous observations [5]. No such gender difference in plasma fibrinogen level was apparent in the normotensive group. The higher level of fibrinogen in the male may therefore partly explain the higher incidence of thromboembolic complications in hypertensive males [22]. A rise in both plasma fibrinogen concentration and plasma viscosity has also been observed as changes accompanying human aging [23].

The increased platelet aggregation observed in the hypertensive subjects used in this study is not unexpected, as similar observations have been made in the past [9,10]. It has been suggested that enhanced platelet activation may be involved in the acceleration of hypertensive arteriovascular damage and atherosclerosis [7]. Antiplatelet agents have been shown to be effective in reducing vascular events in hypertensive patients and in survivors of ischaemic stroke [24], and with blood pressure reduction in hypertensive patients, there is significant reduction of all platelet hyperactivity parameters [11]. It has been suggested that the inhibitory control of platelet aggregation in normal subjects is lost in hypertensive subjects [25]. Beta-endorphin seems to be involved in the inhibition of platelet aggregation in normal subjects [24]. Results of in vitro platelet aggregation studies do not necessarily become applicable to in vivo activity, but studies on plasma concentrations of platelet-specific proteins [7-10] have led to the belief that there is enhanced intravascular platelet aggregation in hypertensive subjects. The increased platelet activation is an indicator of vascular complication in hypertension. This may be primary rather than secondary as it is apparent from this study that platelet aggregation is increased in hypertensive subjects who are without apparent vascular complications. The observation of Guicheney *et al* [8] that there is no significant in vivo platelet activation in hypertensive subjects devoid of cardiovascular complications might suggest that it is a secondary phenomenon.

The combination of increased plasma viscosity and increased platelet aggregation in hypertensive patients may act to increase the risk of thrombogenesis and atherosclerosis, leading to a state of hypoperfusion and impaired microcirculation. The fact that the patients used in this study were without any apparent complication in spite of the abnormalities in rheologic factors and markers of thrombogenesis seems to lend credence to the observations of Vaya *et al.* [17] who noted that in normotensive offspring of hypertensive patients the haemorheological alterations might play a role in the pathogenesis of atherosclerotic process. The identification of haemorheological abnormalities prior to the development of complications of hypertension may suggest the possible primary prevention of the development of thromboembolic complications in hypertensive patients, with early institution of appropriate therapy. There have been conflicting reports on the effect of antihypertensive therapy on haemorheology and platelet aggregation. Angiotensin converting enzyme inhibition, with Ramipril [4] and calcium channel blocking with Isradipin [4], amlodipine [26] and nitrendipine [27], have been shown to have beneficial effects on blood rheology. Metoprol also has beneficial effects on blood viscosity [26], while increase in blood viscosity was observed on administration of hydrochlorothiazide [27]. Prazosin therapy was recently shown to be ineffective in the correction of increased platelet aggregation [9], whereas all platelet hyperactivity parameters were shown to be reduced significantly with the normalization of blood pressure with the Angiotensin converting enzyme inhibitor, quinapril, alone or in combination with the calcium antagonist, nifedipine [11].

This study suggest that thrombogenic and rheologic factors are abnormal in Nigerian hypertensive patients even before the appearance of complications. It is possible that early institution of appropriate and effective antihypertensive therapy may reduce or even abolish thomboembolic complications, which are common in these patients.

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