Haemorheologic and fibrinolytic activity in Nigerian HIV infected patients.

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Abstract

Objective: Human immunodeficiency virus (HIV)-infected patients, especially those on antiretrovirals are at risk of cardiovascular disease (CVD). The haemorheologic and fibrinolgtic activity of treatment naïve Nigerian HIV-infected patients were investigated.

Methods: Blood was collected from 50 newly diagnosed treatment naïve HIV-infected patients and 50 apparently healthy HIV seronegative individuals that served as controls. Haematocrit values, plasma and serum viscosity, plasma fibrinogen concentration and euglobin lysis time were determined.

Result: The mean ± standard deviation of haematocrit value of HIV infected patients (31.70 ± 6.33%) was significantly lower ($p<0.0001$) than those of controls (39.50 ± 2.43%). The plasma serum viscosity, plasma fibrinogen concentration and euglobin lysis time of HIV-infected patients were significantly higher compared with those of controls ($p<0.0001$).

Conclusion: Treatment naive Nigerian HIV-infected patients have a defective blood flow and fibrinolytic system, which may predispose them to CVD.

Key words: Haemorheology, Fibrinolytic activity, HIV, Cardiovascular disease, HIV Treatment naïve, Nigerian.

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Intrdouction

Human immunodeficiency virus (HIV)-infected patients have been reported to be at risk of cardiovascular diseases (CVD), mostly due to the use of antiretroviral agents [1,2]. Protease inhibitors have been mostly implicated[3]. All these are also connected to fat redistribution dyslipidaemia and insulin resistance observed in HIV patients [4,5].

Plasminogen activator inhibitor-1 (PAI-1) and tissue type plasminogen activator (tPA) antigens, markers of inpaired fibrinolysis and increased CVD risk, are increased in association with hyperinsulinaemia in patients with HIV and fat redistribution[1]. This indicates that the CVD observed among HIV patients is connected to fibrinolytic activity. Also, it has been reported that HIV-infected patient have a defective blood flow system [6], thus predisposing them to CVD.

Being of African origin has been reported as a risk factor for anaemia among HIV-infected patients[7], thus in this study, we report on the haemorheologic and fibrinolytic activity of treatment naive Nigerian HIV-infected patients.

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Materials and methods

Study Population:

A total of 100 subjects consisting of 50 newly diagnosed treatment navie HIV positive patients and 50 apparently healthy HIV sero-negative individuals that served as controls were used in this study. The HIV patients were attending the heart-to-heart clinic in Central Hospital Benin City. Verbal informed consent was obtained from all subjects (HIV positive and controls). The controls were matched with the HIV patients for age and gender. The protocol for this study was approved by the Ethical Committee of Central Hospital Benin City, Nigeria.

Ten milliliter of blood was obtained from each subject and 4.5ml was dispensed into a 0.5ml 3.8% sodium citrate container. The remaining blood sample was dispensed into a plain container, allowed to clot and the serum used for serum viscosity test.

Determination of Haematocrit Values and Plasma Fibrinogen Concentration.

Haematocrit values were estimated manually while the plasma fibrinogen concentration was estimated using the method of Millar *et al* (8). Briefly, two microhaematocrit

tubes were about three-quarters of their length filled with citrated blood and sealed on one end following centrifugation for 5mins at 12,000g (Hawksley, haematocrit centrifuge; Hawksley, England), the haematocrit values is read with a Hawksley haematocrit reader. The capillary tubes were then placed inside a water bath at 56% for 3mins and re-centrifuaged for 3mins at 12,000g. The thickness of the precipitated fibrinogen was measured under a binocular lens microscope fitted with a calibrated eyepiece micrometer. The fibrinogen concentration (g/L) was estimated as the ratio of fibrinogen precipitate to the total height of plasma plus fibrinogen and multiplied by 100. The mean of the 2 tubes was taken.

Determination of Plasma and Serum Viscosities.
A modification of the method of Reid and Ugwu (9) was used. A 1ml syringe with a hypodermic needle (21.6 x 0.8 x 4mm) was used. Briefly, plasma or serum was drawn into the syringe, avoiding or bubbles, till the 1.0ml mark. The plunger was carefully, removed and the time taken for the entire plasma or serum to drain was noted. This was done twice for each sample and the average taken for that sample. The entire process was repeated using distilled water. The plasma or serum viscosity is the ratio of the flow rate of plasma or serum to that of water.

Determination of Euglobin Lysis Time.
The method described by Omoigberale *et al.* (10) was used Briefly, to 9.5ml of 1% acetic acid in a test tube, 0.5ml of citrated plasma was added and the tube kept at 4^0C for 30mins to precipitate the euglobin fraction. The tube is then centrifuged at 2000rpm for 10mins. The supernatant is discarded and the tube inverted to remove all acetic acid. The deposit was reconstituted with 0.5ml borate buffer (sodium borate, 1g, sodium chloride, 9g and distilled water, 1000ml). The tube was pre-warmed at 37^0C alongside calcium chloride (0,025M) for 2mins, and 0.5ml of the calcium chloride was added to the tube containing the deposit and borate buffer. A stop watch was started immediately a clot was observed and the time taken for the euglobin fraction to lyse completely was recorded in minutes.

Statistical Analysis
The data were analyzed using unpaired student t-test.

Result
The range, mean ± standard deviation of haematocrit values of HIV-infected patients and controls were 16-45%, 31.70 ± 6.33% and 35-46%, 39.50 ± 2.43% respectively. This differences was statistically significant ($p<0.0001$). The plasma viscosity serum viscosity, plasma fibrinogen concentration and euglobin lysis time were significantly higher than those of controls ($p<0.0001$) (Table 1).

Table 1: Comparison of haematologic parameters in HIV infected subjects and controls

	HIV+ve sujects (n=50)		Controls (n=50)		
Parameters	**Range**	**$\bar{X}$ ±S.D (95%CI)**	**Range**	**$\bar{X}$ ±S.D (95%)**	**p-value**
Haematocrit (%)	16-45	1.70 ± 6.33 (30.31, 33.01)	35-46	39.50 ± 2.43 (38.17, 40.87)	P < 0.0001
Plasma Viscosity	1.21-3.59	1.98 ± 0.34 (1.91,2.05)	1.06-1.35	1.22 ± 0.08 (1.16, 1.29)	P < 0.0001
Serum Viscosity	0.98-4.02	1.94 ± 0.55 (1.83, 2.05)	1.06-1.48	1.24 ± 0.09 (1.13, 1.36)	P < 0.0001
Plasma Fibrinogen Concen. (g/L)	1.33-9.67	5.13 ± 1.90 (4.74, 5.52)	2.00-4.00	2.90 ± 0.52 (2.51, 3.29)	P < 0.0001
Euglobinlysis Time (mins)	243-1440	544 ± 401 (462.50, 624.90)	115-274	189 ± 37.70 (105.70, 273.20)	P < 0.0001

$\bar{X}$ = Mean, S.D = Standard deviation, CI = Confidence interval.

Discussion

In this study HIV-infected treatment naive patients had a significantly lower haematocrit value than controls ($p<0.0001$). This observation is in line with earlier reports[7,11]. The haematocrit value affects the enythrocyte sedimentation rate which in turn affects enythrocyte aggregation and ultimately blood flow[6]. Thus, HIV patients may be at risk of CVD. Haematocrit values of HIV patients are usually used to check disease progression and quality of life[7]. The introduction of the highly active antiretroviral therapy (HAART) has seriously improved haematocrit values and quality of life of HIV patients[11]. Thus, the placement of these patients on HAART may improve their haematocrit values. The increase in plasma viscosity observed among HIV patients in this study correlates with the observed increase in plasma fibrinogen concentration. Fibrinogen concentration significantly affects plasma viscosity. These

findings agree with earlier reports[6,12]. It has been reported that increase in fibrinogen concentration (and by extension plasma viscosity) is a risk factor for CVD [13]. Increase in serum viscosity has similar effect. Both plasma and serum viscosity are useful indicators of acute inflammation. Increase in inflammatory proteins such as haptoglobin, c-reactive proteins and immunoglobulins produced against the HIV virus or any other opportunistic infection associated with HIV, cause an increase in serum viscosity.

Impaired fibrinolysis is a risk factor for CVD. Fibrinolytic activity can be measured either by determining the euglobin lysis time (ELT) or the concentrations of PAI-1 and tPA, etc. In this study, the ELT was measured and found to be significantly higher ($p<0.0001$) in HIV patients than controls. This means a lower or defective fibrinolytic activity in HIV patients. Increase in both PAI-1 and tPA have been reported in HIV patients[1]. In the ELT method used in this study, the euglobin consist of tPA, plasminogen and fibrinogen. [10] tPA antigen assays measure both free tPA and inactive tPA complexed with PAI-1(1). If follows therefore, that the defective fibrinolytic system in HIV patients is due to very low levels of free tPA.

These findings suggest a likely problem in management of HIV patients, as some antiretrovirals, though improving haematocrit value, predispose to CVD. Those in the management of these patients should bear in mind the associated risk of CVD.

In conclusion, this study reveals that treatment naïve Nigerian HIV patients have a defective blood flow and fibrinolytic system, which are important risk factors for CVD.

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