Antiretroviral Therapy and Haemostatic Parameters in HIV Patients with Haemoglobin Phenotypes AS and AA

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Abstract

Background: *Haematological and coagulation defects have been reported in advanced HIV infection, the occurrence of the S gene is high in African Blacks. HIV infection is on the increase in Nigeria. About a quarter of Nigerians have haemoglobin phenotype HbAS. The haemostatic parameters have not been fully determined in Nigerians with haemoglobin HbAS*

Aims: *To determine the effects of HIV infection on haematological and haemostatic profiles of Nigerians with the sickle cell trait : haemoglobin As(HbAS) and haemoglobin AA(HbAA)*

Methods: *Subjects comprised of phenotype HBAS and HB AA patients who were newly diagnosed with HIV infection and those already on antiretroviral therapy(ART). Control group comprised of apparently healthy subjects with haemoglobin phenotypes AS and AA. Blood samples were analysed for haematological parameters :(haematocrit(Hct), total white cell count (WCC), platelet count(Plt)) and Haemostatic parameters: (plasma fibrinogen concentration(Pfc), prothrombin time(PT) and Activated partial thromboplastin time(APTT) . All analyses were carried out using standard techniques. Quantitative assay was conducted to estimate D-dimer levels.*

Results: *A total of 145 subjects were studied. The mean haemoglobin(Hb), and WCC were higher in HBAS than HbAA subjects with HIV infection but the differences in the mean values were not Statistically significant($p>0.5$). The PT, APTT and the Pfc were significantly more prolonged in HBAS with HIV infection than in Hb AS controls($p<0.0001$). The haemostatic parameters were also significantly more prolonged in HBAA with HIV than HbAA HIV negative controls ($p<0.0001$). Elevation of D-dimer was found in 12 and 20% of HbAS and HbAA HIV positive subjects respectively. Positive D-dimer was found to be significantly associated with HIVinfection ($p = 0.0007$) in both HbAS and HbAA subjects.*

Conclusion: *This study has shown that Hb phenotype does not influence the effect of HIV infection on haemostatic and haematological parameters. Elevated plasma fibrinogen concentration, prolongation of APTT and presence of D-dimer, in HIV infection may suggest an inflammatory response as well as subclinical coagulapathy*

Introduction

The HbS gene is prevalent in Sub Sahara Africa with an incidence of 2.6% for haemoglobin genotype SS(HbSS) and 26.8% for heterozygous state(AS) in Nigeria.[1] Individuals homozygous for the S gene had varying clinical manifestations including thrombotic stroke from micro vascular occlusion by sickle cells.

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Infection with the human immunodeficiency virus (HIV) is associated with diverse disease conditions. Haematological and rheological alterations such as low platelet count and elevated fibrinogen have been described in patients infected with the virus[2-6] Haemostatic alterations have also been reported in these patients. A number of potential mechanisms have been proposed to explain the observed hypercoagulability in HIV-infected patients[7]. These include the presence of the anti-phospholipid and anti-cardiolipin antibodies, decreased activities of natural anticoagulants like protein S and increased platelet activation[7] Some studies have shown a correlation between the viral load and haematological parameters in HIV[2,3].

An increased risk of clinical events that were not AIDs related had been described in a large cohort of HIV infected adults after interruption of antiretroviral treatment (ART).[8,9] Many of these events [cardiovascular, kidney and liver diseases] have been found to be associated with impaired fibrinolysis. Although thrombosis was rarely seen in HIV/AIDS patients before the advent of antiretroviral therapy, recent studies have shown an increasing rate of venous thromboembolism in these patients[9,10]. There is fresh evidence from recent literature to support the believe that the introduction of highly active antiretroviral therapy (HAART) has led to reduction in HIV- associated morbidity and mortality[11-13] Previous Studies on HIV/AIDS and antiretroviral therapy were mainly in Caucasians. Antiretroviral drugs are increasingly more available to Nigerians living with HIV/AIDS. Although individuals with Hb phenotypes AS are known to have little or no clinical manifestations under physiological conditions, the effect of the HIV virus on the AS haemoglobin has not been fully determined to our knowledge. There is therefore a need to assess the effects of these haematological parameters and haemostatic parameters on this category of patients

The aim of antiretroviral therapy in HIV is to reduce the viral load and thus the disease progression[11]. Our aim therefore is to compare haemostatic and rheological parameters in treatment naive HIV-positive Hb AS patients and those on antiretroviral therapy.

Subjects and Methods

Subjects: Comprised of newly diagnosed adult patients with HIV without ART and HIV positive subjects already on ART (a combination of 2 reverse transcriptase inhibitor and a protease inhibitor) seen at a Government Hospital involved in HIV/AIDS treatment in Lagos. Apparently healthy HIV negative members of the hospital (age matched with test group) served as controls. Informed consent was obtained from subjects and controls before commencement.

Blood samples: Ten milliliters of venous blood was collected from each patient by clean venu-puncture while avoiding stasis, 4.5ml of blood was anticoagulated with 3.8% sodium citrate solution in a ratio of 9 parts of blood to 1 part of anticoagulant. Five milliliters was decanted into EDTA bottle for haematological studies. Plasma was obtained by centrifuging at 1,500g for 15 minutes at room temperature (25^0C). Plasma fibrinogen was measured by the clot weight method of Ingram[14]. Plasma viscosity and whole blood viscosity were measured using the fluorometric method of Reid and Ugwu as previously documented in the Nigerian Journal of Physiological Sciences[15.] The haematocrit was measured using the microhaematocrit method and the erythrocyte sedimentation rate was measured as described by Dacie and Lewis[16]. The PT and the APTT were performed according to manufacturer's instruction (Cypreas diagnostics Belgium) HC00100 lot: 991215 and HC00200 lot: 980902.

HIV testing was done using ELISA based rapid screening kits. Hb phenotype was determined using Hb electrophoresis machine

Subjects and blood samples were handled using the new guidelines for haemorheological laboratory techniques where applicable[17]. Qualitative assay (ActiScreen ™ XL-FDP (Stamford, USA) an immunoagglutination test for XL-FDP in human plasma) was conducted to estimate D-Dimer levels

Data Analysis: Data were analysed using Instat gragh pad ™ statistical package. Group means were compared using the alternate student t-test. The results are presented as mean ± the SD of the mean. A value of P< 0.05 was considered significant.

Results

A total of 145 subjects (age range 20 -59 years) were studied. They comprised of 25 HIV-positive HBAS, 70 HIV-positive HBAA subjects and 50 HIV negative controls. There were no statistically significant differences in haematological parameters of HIV-positive subjects with Hb phenotypes AS and AS (p>0.5). Haematological and haemostatic parameters were significantly higher in Hb AA subjects compared to controls. PT, APTT and Pfc were also significantly higher in HbAS subjects than AS controls (p<0001)table1.

Table 2.

Haematological, haemostatic and rheological parameters in treatment-naive HIV patients and HIV patient on ART with HB phenotype AS

Parameters	Treatment naive subjects (n= 14)	Subjects on ART (n = 11)
PCV(%)	32.5 ±6.9	34.9 ±3.9^{ns}
Hb(g/dl)	10.9 ±2.3	11.6±1.3^{ns}
WBC (x 10^9 /l)	4.8 ± 1.4	5.6 ± 1.3^{ns}
PLT(x 10^9 /l)	217.1 ±84.9	207.6 ±72.1^{ns}
ESR(mm/Hr)	85.4 ± 46.8	61.6 ±51.1^{ns}
PT(s)	37.3 ±15.7	32.2±5.8^{ns}
APTT(s)	39.9 ±18.6	58.2 ±10.9^{ns}
PFC(g/l)	4.6 ±1.5	3.1±1.2*

*^{ns} = not significant, * = p<0.05*

PCV, packed cell volume. Hb, haemoglobin. WBC, white cell count. PLT, platelet count. ESR, erythrocyte sedimentation rate. PT, prothrombin time. APTT, activated partial thromboplastin time. PFC, plasma fibrinogen concentration.

Table 2 shows the haematological and

Table 1.

Haematological and Haemostatic Parameters in HbAS and HbAA Patients and Controls

Parameters	Hb Phenotype AA		Hb Phenotype AS	
	Controls (n = 42)	Subjects (n = 70	Controls (n = 8)	Subjects (n = 25)
PCV(%)	42.9±5.1	32.1±7.2***	43.1±5.9	33.6±5.7**
Hb(g/dl)	13.9±1.5	10.8±2.4***	13.9±1.7	11.2±1.9**
WBC(x 10^9/l)	5.7±1.1	4.9±1.7^{ns}	6.1±1.1	5.1±1.4^{ns}
PLT(x 10^9/l)	243±69.8	227±83.1***	230±58.7	217.7±78.0^{ns}
ESR (mm/Hr)	4.1±2.7	69.8±42.6***	6.0±4.1	73.0±49.6***
PT(s)	12.6±0.8	32.9±9.1***	13.1±1.2	34.8±12.1***
APTT(s)	33.1±1.2	51.7±18.1***	33.0±1.9	48.7±17.1***
PFC (g/l)	2.8±0.4	4.4±1.1**	2.4±0.6	4.4±1.2***

*ns = not significant, ** = p<0.01, *** = p <0.0001*
PCV, packed cell volume. Hb, haemoglobin. WBC, white blood cell count. PLT, platelet count. ESR, erythrocyte sedimentation rate. PT, prothrombin time. APTT, activated partial thromboplastin time. PFC, plasma fibrinogen concentration.

haemostatic parameters in treatment naive HIV-positive Hb As subjects and those already on ART. There was a significant reduction in pfc (P<0.05) in the treatment naïve group.

Table 3 compares the effect of ART on the parameters studied in HIV-positive AA subjects. The Hct and Hb were significantly higher in those on therapy than treatment naïve group (p<0.0001). the PT and Pfc were significantly lower in the treatment naïve group(p = 0.0427 and P< 0.0001 respectively). Table 4 compares the number of D-dimer positive subjects HIV- positive HbAS and HBAA subjects and controls. About 18% (17/95) of the HIV- positive cases had positive D-dimer (D-dimer >200ng/ml), while 78/% were negative (D-dimer <200ng/ml). This was significant compared to controls 0% (0/50) (p = 0.0007, OR 0.0441). Twelve percent (3/25) of AS subjects were positive for D-dimer while

Table 3.

Haematological, haemostatic and rheological parameters in treatment-naive HIV patients and HIV patient on art with Hb phenotype AA

Parameters	Treatment naive Subjects (n = 36)	Subjects on ART (n = 34)	P-value
PCV(%)	28.8±8.1	35.6 ±3.6	<0.0001
Hb(g/dl)	9.7 ± 2.7	11.9 ±1.2	<0.0001
WBC(x 10^9/l)	4.9 ± 2.1	4.8 ± 1.1	0.8024
PLT(x 10^9/l)	223.8 ± 80.2	230. ±45.1	0.7394
ESR (mm/Hr)	72.6 ± 40.2	67.2 ±45.1	0.5995
PT(s)	34.8 ±8.6	30.5 ±9.2	0.0477
APTT(s)	50.4 ±18.2	53.4 ±18.1	0.4918
PFC (g/l)	4.4 ±1.2	2.9±1.2	<0.0001

PCV, packed cell volume. Hb, haemoglobin. WBC, white blood cell count. PLT, platelet count. ESR, erythrocyte sedimentation rate. PT, prothrombin time. APTT, activated partial thromboplastin time. PFC, plasma fibrinogen concentration.

Table 4.

HIV Posisitive HbAS and HbAA subjects and D-Dimers

Study group	D-dimer positive (>200ng/ml) (%)	D-dimer negative (>200ng/ml) (%)	Total (%)
Control	0(0)	50(34.5)	50(34.5)
HbAS	3(2)	22(15.2)	25(17.2)
HbAA	14(9.7)	56(38.6)	70(48.3)
Total	17(11.7)	128(88.3)	145(100)

HbA and HbAA vs control: p = 0.0007, OR = 0.0441HbAs vs HbAA: p = 0.5455, OR = 0.5455

20% (14/70) of AA were positive. There was no statistically significant difference between the percentage positivity in AS and AA subjects (p= 0.5455, OR = 0.5455).

Discussion

In this study, a significant reduction in haemoglobin concentration was found in HIV-positive HbAS and HBAA subjects compared to the HIV-negative control, anaemia has been reported in HIV infection[2,3]. A number of reasons have been canvassed to explain the anaemia of HIV infection they include: reticuloendothelial iron block, impaired response to erythropoietin and marrow infiltration by lymphomas and effect of ART[18]. One or two of these mechanisms might have been responsible for the low haemoglobin concentration in both HbAS and HbAA subjects.

A significantly lower platelet count was also found in this study in HbAA HIV-positive groups compared with HbAA controls; this is however not so in HIV - positive HbAS subjects the difference may be due to the relatively small population of the HBAS group. In a previous study, thrombocytopaenia has been described as the initial presentation in about 10% of cases [19].Significantly this study did not show any difference in the haematological and haemostatic parameters between HBAA and HbAS subjects, though the small population of HIV-positive AS subjects studied may not really support this claim, we believe that the strength of the association when compared with AS controls(P<0001) should not be overlooked and may further support the existing believe that the sickle cell trait(HbAS) is a benign condition.

A significant increase in plasma fibrinogen was found in both HIV-positive HB AS and HbAA subjects. Expectedly, the plasma fibrinogen concentration was significantly higher in treatment-naive subjects, fibrinogen, an acute phase protein together with other inflammatory markers have been reported to be higher in HIV infection[20]. The increase may be due to the presence of inflammatory cytokines; in addition, the higher fibrinogen concentration in both HbAA and HbAS HIV-positive subjects may further suggest an enhancement of coagulation in HIV infection. However, the prolongation of APTT, PT, together with a decrease platelet count in our HIV-positive subjects, simulate sub-clinical disseminated intravascular coagulopathy in some of these subjects.

Positive D-dimer was found in 12%(3/25) of HbAS and 20%(14/70) of HbAA subjects this was significant when compared with controls (p = 0.0007). The finding of elevated of D-dimer in some HIV positive subjects is corroborated by earlier studies, where an increased D-dimer level was reported[21,22.] This observation has made HIV to be regarded as a prothrombotic condition. Lewis etal[8] had reported an increased in D-dimer together with the elevation of other inflammatory markers on suspension of antiretroviral therapy. They hypothesized that elevated D-dimer levels and interlukin-6, suggest HIV-infected patients at the risk of death. Indeed the magnitude of the association seen between HIV infection and D-dimer(p = 0.0007) in this study, despite our small sample size is an indication that further studies to ascertain the exact effect of elevated D-dimer in HIV positive Nigerians

Our study also revealed a higher haematocrit and a significant in HIV-positive HbAA subjects on therapy compared to treatment naive group, this finding is contrary to an earlier observation that ART contribute to the anaemia in HIV-positive patients on therapy[18]. It is however plausible that the effect of the drugs is more devastating in those with advance HIV infection since this study did not assess parameters in relation to viral load or disease severity. It may be necessary to test this hypothesis in subsequent studies of HIV-positive Nigerians. Moreover these parameters have been shown to be correlated with the viral load in a previous study[8].

The activated partial thromboplastin time were significantly more prolonged in both HbAA and

HB AS subjects compared to controls this agrees with earlier reports where a prolongation of the APTT was obtained in HIV subjects[21,22], the presence of the lupus anticoagulant has been suggested as a likely reason for this prolongation[22]. Similarly we also observed a prolonged PT in both study groups, the combined prolongation of the PT and the APTT would suggest a global disturbance of haemostatic factors in these groups of patients.

In conclusion, we found, higher plasma fibrinogen concentration, thrombocytopaenia, and prolongation of the APTT and PT in both treatment- naive and HIV-positive HbAA and HbAS subjects on therapy. This may suggest a subclinical coagulopathy in HIV- positive patients. While the increased D-dimer levels in some of these subjects further supports the existing literature that HIV infection is a prethrombotic state. This study also shows that Hb phenotype does not influence the effect of HIV infection on haemostatic and haematological parameters.

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