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Full Length Research Article

Prevalence of Antibodies to HAART Agents among HIV Patients in Benin city, Nigeria

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ABSTRACT

Against the background that some human immunodeficiency virus (HIV)-infected patients on the highly active antiretroviral therapy (HAART) still experience anaemia, some possible reasons for this were investigated. Blood was collected from 50 newly diagnosed treatment naive HIV-infected patients, 100 HIV-infected patients on HAART and 30 apparently healthy HIV seronegative individuals that served as controls. Haematocrit values, red blood cell distribution width (RDW) and the presence of antibodies to the drugs in the HAART regimen were determined. The mean \pm standard deviation of haematocrit values of HAART naive HIV patients ($36.90 \pm 5.61\%$) and those on HAART ($37.20 \pm 6.20\%$) were significantly lower compared to controls ($41.50 \pm 5.88\%$) ($p = 0.001$), though the difference between HIV patients on HAART and those that were HAART naive was not significant ($p = 0.836$). Although, the RDW of HIV patients were lower than controls, the difference was only significant ($p = 0.026$) between controls ($17.00 \pm 6.01\%$) and HAART naive HIV patients (15.00 ± 1.87). A total of 81 (81%) out of the 100 HIV patients on HAART had antibodies to one or more of the HAART drugs. Antibodies to nevirapine (58%) was higher compared to stavudine (44.6) and zidovudine (42%) ($p = < 0.05$). There was no significant improvement in haematocrit of HIV patients on HAART over HAART naive HIV patients. Use of zidovudine, nutritional deficiency and presence of antibodies to the HAART drugs may have been responsible

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Key words: antiretroviral therapy, haematocrit, human immunodeficiency virus

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INTRODUCTION

Highly active antiretroviral therapy (HAART) therapy entails treatment with a combination of two nucleoside reverse transcriptase inhibitors and a potent protease or non-nucleoside reverse transcriptase inhibitors, and has generally been taken as the gold standard for management of HIV patients (Odunukwe *et al.*, 2005). However, serious interactions with metabolism of sugar and lipids lead to the appearance of well documented disorders such as insulin resistance, abnormalities in lipid metabolism and lipodystrophy, particularly associated with protease inhibitors (Andrade and Cotter, 2006). This leads to the decline in the use of protease inhibitors in the HAART regimen. HAART have been reported to improve haematocrit values of human immunodeficiency virus (HIV) infected patients, despite documented evidence that these antiretroviral drugs used as monotherapy have cytopenic effect (Odunukwe *et al.*, 2005). HAART have also been reported to significantly reduce the morbidity and mortality of HIV infection (Gea-Banacloche and Lane, 1999). Nevertheless, in the early 2000s adverse reactions began to appear and started to challenge the goals of HAART (Andrade and Cotter, 2006). Mildvan (2003) reported that many HIV positive patients receiving HAART still develop mild to moderate anaemia. The use of zidovudine, alone or in the HAART combination has been reported to be associated with anaemia (Moyle, 2002). Inhibition of haemoglobin synthesis and globin gene transcription have reported as the mechanism by which zidovudine cause anaemia (Wood *et al.*, 1992), and this could be responsible for this observation.

Anaemia is also known to be caused by drugs, through various mechanisms. One of which is production of antibodies against the drug or its metabolite. The mechanism involves the drug or its metabolite attaching to the surface of red blood cells and this triggers antibody production. The antibody produced binds to the drug on the red cell membrane and the complex activates complement with resultant cell lysis, or the sensitized cells do not activate complement, but are destroyed extravascularly by the reticulo-endothelial

system (Pertz, 1986). The purpose of this study was 1) to determine the haematocrit and red cell distribution width (RDW) of HIV patient (on HAART and not on HAART) compared with healthy controls, and 2) to determine the presence of antibodies to the drugs in the HAART regimen.

MATERIALS AND METHODS

Study Population

The study was approved by the Ethical Committee of the University of Benin Teaching Hospital. Ten milliliters of blood was collected each from 50 newly diagnosed treatment naïve HIV positive patients (17 males and 33 females), 100 HIV-Infected patients on HAART for 3-6 months (39 males and 61 females), and 30 age and gender matched apparently healthy HIV seronegative individuals (13 males and 17 females) that served as controls. The HIV patients were attending HIV clinics in the University of Teaching Hospital (UBTH) Benin City, Nigeria. Verbal informed consent was obtained from every subject used in this study. Five milliliters of blood was dispensed in ethylene diamine tetracetic acid (EDTA) containers and the remaining into plain containers. The serum obtained from clotted samples in the plain container were frozen at -20°C until needed. The HAART regimen for HIV patients on HAART consist of zidovudine, stavudine and nevirapine.

Determination of Haematocrit and RDW

Haematocrit values and RDW of all samples were determined using an autoanalyser – Sysmex KX – 21 (Sysmex Corporation, Kobe, Japan).

Detection of Antibodies to HAART Drugs

A modification of the method described by Petz (1986) was used. Briefly, one tablet each of zidovudine (Retrovir®, Glaxosmithkline, Spain), stavudine (Zerit®, Bristol-Myers Squibb, France) and nevirapine (Viramune® Boehringer Ingelheim, Germany) was dissolved in 10ml of normal saline in 3 separate test tubes (all drugs were freely donated by Pharmacist Ekiuwa Eribo, Pharmacy department, UBTH). The drug

suspension was centrifuged and the supernatant was used in the test. Equal volume of 3% washed group O rhesus D negative red cells and the supernatant of zidovudine solution were placed in a test tube and incubated at 37°C for 30mins. The drug – red cell mixture was washed 4 times with normal saline to remove excess drug. Equal volume of study subjects sera and drug – red cell mixture were placed inside a clean test tube and incubated at 37° for 1 hour. The mixture was centrifuged after incubation and agglutination or haemolysis was watched out for. If negative, the mixture was washed 4 times and antihuman globulin (Plasmatec Laboratories Limited, United Kingdom) was added. This was followed by centrifugation and agglutination or haemolysis watched out for.

Controls were performed in the same way with 1) subject serum and group O red cells without drugs, and 2) drug red cell complex without subjects serum (both gave negative results).

The above procedure was repeated using stavudine and nevirapine separately.

Statistical Analysis

The parametric data obtained were analyzed using student t-test. The non parametric data were analyzed using Chi square(X²) test.

RESULTS

The haematocrit of HIV patients were significantly lower than controls (p=0.001), but did not differ significantly (p=0.836) between HIV patient on HAART (with mean ± standard

deviation of 37.20 ± 6.20%) and does not on HAART (mean ± SD of 36.90 ± 5.61%) (Tables 1 and 2). Although, the RDW of HIV patients were lower than controls, the difference was only significant (p=0.026) between controls (mean = 17.00%) and HAART naïve HIV patients (mean = 15.00%).

A total of 81(81%) out of the 100 HIV patients on HAART had antibodies in their serum to one or more of the HAART agents. The prevalence of antibodies to nevirapine (58%) was significantly (X²=6.09, P<0.05) higher compared with stavudine (44%) and zidovudine (42%) (Table 3).

Presence of antibodies to more than one of the HAART agents was significantly higher (X²=5.63, P<0.025) than to one of the agent alone (43% Vs 38% respectively) (Table 4).

HIV patients not on HAART and controls did not show the presence of antibodies to HAART drugs in their sera.

DISCUSSION

Anaemia is one of the haematologic complications seen in HIV-infected patients (Moyle, 2002). HAART has been reported to improved haematocrit values of HIV- infected patients (Odunukwe *et al.*, 2005). Results from this study showed a significantly higher haematocrit of control compared with HIV patient - both on HAART and HAART naïve (p = 0.001) (Table 1 and 2). The cause of anaemia in HIV patients are multifactorial. From this study, three possible reasons were examined.

Table 1:
Haematocrit Values and RDW Of HIV-Infected Patients And Controls.

PARAMETER	HIV PATIENTS				CONTROLS(n =30)	
	HAART Naïve (n = 50)		On HAART (n = 50)		Range	X ± SD (95% CI)
	Range	X ± SD (95% CI)	Range	X ± SD (95% CI)		
PCV (%)	24.40-48.90	36.90 ± 5.61 (35.27,38.63)	19.60-50.90	37.20 ± 6.20 (35.98, 38.35)	30.00-54.80	41.50 ± 5.58 (39.32,43.68)
RDW (%)	12.60-20.10	15.00 ± 1.87 (14.21, 15.71)	12.10-35.00	15.80 ± 3.02 (15.25, 16.31)	11.10-36.80	17.00 ± 6.01 (15.61, 18.43)

RDW = Red Cell distribution width, HAART = Highly active antiretroviral Therapy; X ± S.D = Mean ± Standard deviation, CI = Confidence interval.

Table 2:
Statistical Comparison of Haematocrit Values and RDW of HIV Patients and Controls

Parameters	On HAART Vs HAART Naive	On HAART Vs Controls	HAART Naive Vs Controls
PCV (%)	P = 0.836	P = 0.001	P = 0.001
RDW (%)	P = 0.081	P = 0.129	P = 0.026

Table 3:
Prevalence of Antibodies to HAART Agents Among HIV Patients On HAART (N = 100)

HAART Agents	Presence of antibodies	Absence of antibodies
Zidovudine	42 (42%)	58 (58%)
Stavudine	44 (44%)	56 (56%)
Nevirapine	58 (58%)	42 (42%)

n = number of HIV patients on HAART
 $\chi^2 = 6.09$, $p < 0.05$

Anaemia associated with HIV infection has been attributed to bone marrow suppression (Moyle, 2002; Odunukwe *et al.*, 2005). This may explain the lower haematocrit to HIV-infected patients. Haematocrit of HIV-infected patients on HAART did not differ significantly from those that were HAART naïve ($p=0.836$) (Table 1 and 2). This finding is not in agreement with those of Odunukwe *et al.* (2005) and Belperio and Rhew (2004). The difference between our result and that of Odunukwe *et al.* (2005), could be due to the use of zidovudine as one of the HAART agents by the HIV patients on HAART in this study. Lamivudine replaced zidovudine in Odunukwe *et al.* (2005) study. This is important as the use of zidovudine alone or in combination has been reported to be associated with anaemia (Moyle, 2002). Inhibition of haemoglobin synthesis and globin gene transcription and toxicity to bone marrow cells, particularly, erythroid lines have been suggested as possible mechanisms of anaemia by zidovudine (Cretton *et al.*, 1991; Weidner *et al.*, 1992). Thus, the suggestion of Moyle (2002) that HIV-infected patients of African origin (a risk factor for anaemia in the HIV era) should avoid zidovudine, may be valid.

Nutritional deficiencies have been reported to be among the factors that play a role in the development of anaemia among HIV patients (Volberding, 2000; Odunukwe *et al.*, 2005). Traditional red blood indices, such as MCV, are used to assess nutritional deficiency. However, increase MCV in HIV-treated patients often reflects the use of zidovudine (Moyle, 2002) and not necessarily nutritional deficiency. This limits the use of MCV to assess nutritional deficiency as the patients in this study took zidovudine as part of their HAART drugs. The RDW has been recommended as a biomarker to assess nutritional status. (Romero *et al.*, 1999). HIV-infected patients irrespective of whether they were on HAART or not, have lower mean RDW compared with controls (Table 1), although this difference is only significant between HAART naïve HIV-infected patients and controls ($p = 0.026$) (Table 2). Perhaps, nutritional deficiencies - a possible cause of anaemia (Volberding, 2000), exist in our HIV patients and may contribute to the lower haematocrit observed. Wood *et al.* (2002) reported inadequate nutrient intake among a large proportion of their HIV positive patients. This may also explain the low RDW among HIV patients in this study. There is evidence that nutritional intervention assists in maintaining and optimizing nutritional status and immune function, prevents the development of nutritional deficiency, loss of weight and lean body mass, promotes response to medical treatment and increases longevity in HIV-infected persons (Oguntibeju *et al.*, 2006). Also, nutritional supplementation has been shown to decrease viral load (Bouic *et al.*, 2001; Oguntibeju *et al.*, 2006). Thus, the HIV patients in this study may benefit from nutritional supplements.

Anaemia can also result from red cell destruction as a result of antibodies produced against drugs or their metabolites. A total of 81 (81%) out of the 100 HIV patients on HAART had antibodies to one or more of the HAART drugs with the prevalence of antibodies to nevirapine being significantly higher compared to the other HAART drugs ($p < 0.05$) (Table 3). It was also observed that antibodies to more than one of the HAART agents among the HIV patients on HAART was significant ($p < 0.025$) (Table 4).

The mechanism by which the antibody produced against the drugs causes anaemia involves the drug or its metabolite attaching to the surface of red blood cells and this triggers antibody production. The antibody produced binds to the drug on the red cell membrane and the complex activates complement with resultant cell lysis, or the sensitized cells do not activate complement, but are destroyed extravascularly by the reticulo-endothelial system (Pertz, 1986). It is possible that the non-significant difference in haematocrit between HIV patients that are HAART naive and on HAART (Table 1 and 2) may be due to the presence of these antibodies. However, this will require further investigation.

The severity of HIV infection also influences anaemia. However CD4 count and viral load, which are used to measure HIV progression and severity of infection, were not done because they were not the primary focus of this study. Further studies are needed to determine their relationship with presence of antibodies to HAART drugs.

In summary, in this study, we did not observe any difference in haematocrit between HIV-infected patients on HAART and those that were HAART naive. Use of zidovudine nutritional deficiency and presence of antibodies to the HAART drugs were observed as possible contributing factors. However, the observation of presence of antibodies to the HAART agents, as contributing to anaemia in HIV patients, will require further investigation.

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