

# Prevalence of HIV-related autoimmune haemolytic anaemia in Lagos, Nigeria

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## ABSTRACT

**Background:** Despite a high frequency of anaemia, a positive direct antiglobulin test (DAT) and bone marrow hyperplasia HIV-infected patients, lack of reticulocytosis may cause underdiagnosis autoimmune haemolytic anaemia (AIHA) in them. This study was carried out to determine the prevalence of autoimmune haemolytic anaemia in HIV-infected patients and to compare the haematological/immunological characteristics of subjects with anaemia and those without. **Materials and Methods:** A total of 350 HIV-infected subjects attending the Lagos University Teaching Hospital who consented were recruited for the study. This included 250 subjects with anaemia (haemoglobin concentration <10 g/dl) as cases and 100 subjects without anaemia as controls. Five milliliters of venous blood drawn from each subject was used for the full blood count, reticulocyte count and DAT. **Results:** Subjects with anaemia had lower mean CD4 cell count (284.3 cells/ $\mu$ l) and higher mean reticulocyte per cent (1.5%) than the non-anaemic subjects. The frequency of reticulocytosis was higher in female subjects than in males. Only 0.8% (2 of 250) of the study group screened positive to DAT,  $p = 0.0339$ . None of the subjects in control group screened positive to DAT. **Conclusion:** Autoimmune haemolytic anaemia is a rare complication of HIV infection in our geographical location.

**Key words:** Autoimmune haemolytic anaemia, direct antiglobulin test, haemoglobin, reticulocytosis

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## INTRODUCTION

The prevalence of human immunodeficiency virus (HIV) among adults (above 14 years) in Nigeria is estimated to be 3.6%.<sup>1</sup> With a population of approximately 150 million, 3.3 million adults lived with HIV/AIDS in Nigeria at the end of 2009.

Patients infected with HIV have one thing in common; a deficit in cell mediated immunity, resulting predominantly from a significant reduction in the number of circulating CD4+ T cells. The mechanism by which the virus depletes these cells is not clearly understood and remains one of

the most fundamental and controversial issues in AIDS research. A plausible mechanism involves binding to, and signaling through, CD4 and chemokine receptor molecules on resting CD4 lymphocytes and other cell types of HIV, inducing upregulation of L-selectin and Fas. On returning to blood, these resting HIV-signaled CD4 cells home very rapidly back to peripheral lymph nodes and axial bone marrow where they are subsequently induced into apoptosis.<sup>2</sup>

HIV-infected patients may also develop haematological disorder. This disorder commonly affects all the three cell lines causing anaemia, leucopenia and thrombocytopenia with bone marrow dysplasia being the most common pathology encountered in such patients.<sup>3</sup>

Anaemia in HIV infection may be caused by the same three basic mechanisms that cause anaemia in the general population: Decreased production of red cells due to nutritional deficiency of iron, folic acid and vitamin B12; renal disorders such as HIV-associated nephropathy (HIVAN) causing decreased production of

Access this article online	
Quick Response Code:	Website: www.nigeriamedj.com
	DOI: 10.4103/0300-1652.128175

endogenous erythropoietin; bone marrow suppression by proinflammatory cytokines (IL-1 and TNF) and antiretroviral drugs such as zidovudine; increased red cell destruction due to autoantibodies causing autoimmune haemolysis, haemophagocytic syndrome and disseminated intravascular coagulation; blood loss that may be associated with neoplastic diseases such as Kaposi sarcoma in the gastrointestinal tract or with gastrointestinal lesions that accompany opportunistic cytomegalovirus infection and severe immune-mediated thrombocytopenia.<sup>4</sup>

The haemolysis in AIHA is mainly extravascular, but complement-mediated intravascular haemolysis may occur. The usual laboratory features include reticulocytosis and a positive direct antiglobulin test (DAT) which detects the antibody coating the red blood cell surface.<sup>5</sup> The blood film exhibits polychromasia and spherocytosis, the latter, a hallmark of the disease.

Despite the high frequency of anaemia and a positive DAT in HIV-infected patients, lack of reticulocytosis despite bone marrow hyperplasia may lead to underdiagnosis of AIHA.<sup>6,7</sup>

Reports from Africa had shown that AIHA is very rare.<sup>8-10</sup> The rare occurrence may be due to the fact that acquired autoimmune disorders are not common in many parts of Africa and according to Salawu and Durosini (2002), Africans that develop autoimmune diseases may have hereditary predisposition.<sup>8</sup> This view was also supported by Volberding *et al.*<sup>9</sup> and Olayeni *et al.* who reported a frequency of 3.06% in Benin City, Nigeria.<sup>10</sup>

The aim of this study was to demonstrate the frequency of AIHA in a cohort of adult Nigerian HIV-infected subjects attending the AIDS Prevention Initiative Nigeria (APIN) Clinic of the Lagos University Teaching Hospital (LUTH) and to see if the presence or not of AIHA is related to the severity of the disease with regard to the CD4<sup>+</sup> cell count and haemoglobin concentration.

The aims of this study were (1) to determine the prevalence of autoimmune haemolytic anaemia in HIV-infected patients, and (2) to compare the haematological and immunological characteristics of subjects with anaemia and those without.

## MATERIALS AND METHODS

This study was carried out among HIV-infected subjects attending the Lagos University Teaching Hospital (LUTH), Nigeria, after obtaining an informed consent from each participant. Ethical approval was obtained from the Health Research and Ethics Committee of the hospital.

Inclusion criteria included patients with seropositivity for HIV 1 or 2, and haemoglobin concentration <10 g/dl for cases and >10 g/dl for controls.

All subjects who refused to give consent or were aged less than 14 years were excluded from the study.

About 5 mls of venous blood was drawn from each subject into sodium ethylene diamine tetra-acetate (EDTA) specimen bottles. This sample was used to measure full blood count parameters by Sysmex Analyzer model KX-21N, made by Sysmex Corporation, Kobe, Japan, and for reticulocyte count and DAT. Subjects' demographic data were recorded and CD4<sup>+</sup> cell count values were extracted from subjects' records.

Data was analysed using statistical software packages: SPSS for windows (version 11.5: SPSS Inc, Chicago, IL). Descriptive statistics,  $\chi^2$  test and Student 't' test were used as appropriate. The critical level of significance was set at  $p < 0.05$ .

## RESULTS

The demographic characteristics of subjects are as shown in Table 1. The mean age of subjects with anaemia was  $36.3 \pm 0.5$  years and that of subjects without anaemia was  $36.1 \pm 0.8$  years. The age range with the highest number of subjects in both groups was 25-40 years and females dominated the study in the two groups.

Table 2 shows that the mean Hb concentration of subjects with anaemia was  $8.6 \pm 1.1$  g/dl and  $12.4 \pm 1.4$  g/dl in

**Table 1: Demographic characteristics of subjects**

Characteristics	HIV-infected subjects with anaemia	HIV-infected subjects without anaemia
Total no	250	100
Mean age (yrs)	36.3	36.1
Std error of mean	0.5	0.8
Age group (yrs)		
<25	9 (3.6%)	3 (3%)
25-40	171 (68.4%)	36 (67%)
41-50	55 (22%)	22 (22%)
>50	15 (6%)	8 (8%)
Sex		
Male	108 (30.9%)	
Female	242 (69.1%)	
Male		37 (37%)
Female		63 (67%)

**Table 2: Comparison of mean values of haemoglobin, CD4 cell count and reticulocyte% in study group and control group**

	HIV-infected subjects with anaemia			HIV-infected subjects without anaemia		
Total	250			100		
	Hb g/dl	CD <sub>4</sub> cells/ $\mu$	Retic.%	Hb g/dl	CD <sub>4</sub> cells/ $\mu$	Retic.%
Mean	8.6	284.3	1.5	12.4	387.2	1.1
Standard error of mean	0.11	11.55	0.05	0.14	21.37	0.04

those without anaemia. Subjects with anaemia had lower mean CD4 cell count (284.3 cells/ $\mu$ l) and higher mean reticulocyte per cent (1.5%) than the non-anaemic subjects with mean CD4 cell count of 387.2 and mean reticulocyte count per cent of 1.1%.

Table 3 shows the distribution of reticulocyte count per cent, CD4 cell count and DAT among the subjects. The frequency of reticulocytosis (reticulocyte count  $>2.5\%$ ) was higher in the subjects with anaemia when compared with the control group, as 45 (18%) of the anaemic group had reticulocyte count  $>2.5\%$  compared to 2 (2%) of the non-anaemic group.

Furthermore, more subjects with anaemia had a lower CD4<sup>+</sup> cell count than those without. Of the 250 HIV-positive subjects with anaemia, 81 (32.4%) had a CD4<sup>+</sup> cell count  $<200$  cells/ $\mu$ l and 17% (17 of 100) of the HIV-positive subjects without anaemia had a CD4<sup>+</sup> cell count  $<200$  cells/ $\mu$ l. This difference was, however, not statistically significant,  $p = 0.1041$ . While none of the subjects in control group screened positive to DAT, 0.8% (2 of 250) of the study group, screened positive to DAT.

Association between reticulocyte count and age/sex of subjects with anaemia is shown in Table 4.

There was a higher frequency of reticulocytosis in subjects in the age range of 26-40 years. About 19.3% (33 of 171) of subjects within that age range had reticulocytosis. Furthermore, it was noticed that the frequency of reticulocytosis decreases with age. Only 2 of 9 (22.2%) of the subjects below 25 years had reticulocytosis (reticulocyte count  $>2.5$ ), 16.4% (9 of 55) of subjects within the age range of 41-50 years had reticulocytosis, and 6.7% (1 of 15) of the subjects above 50 years had reticulocytosis. The association between reticulocyte count and age of the subjects was statistically insignificant with  $p = 0.6349$ . The frequency of reticulocytosis was higher in female subjects than in males. About 82.2% (37 of 45) of the female subjects compared to 17.8% (8 of 45) of the males had reticulocytosis. The association between reticulocyte count and sex of subjects was also not statistically significant ( $p = 0.0555$ ).

Table 5 shows association between direct antiglobulin test (DAT) and age of HIV-infected subjects with anaemia. The two subjects belonged to the age groups 41 years and above ( $p = 0.0339$ ). Furthermore, though not statistically significant ( $p = 0.5118$ ), the two DAT-positive subjects were both females but none of them met the criteria that define autoimmune haemolytic anaemia.

Lastly, no significant association was also found between CD4<sup>+</sup> cell count and reticulocytosis. About 16.0% (13 of 81) of subjects with CD4<sup>+</sup> cell count  $<200$  cells/ $\mu$ l had reticulocytosis, while 18.3% (31 of 169) of subjects with CD4<sup>+</sup> cell count  $>200$  cells/ $\mu$ l, also had reticulocytosis.

**Table 3: Distribution of reticulocyte count, CD4 cell count and direct antiglobulin test among the subjects**

	HIV-infected subjects with anaemia	HIV-infected subjects without anaemia		
	Number	%	Number	%
Reticulocyte count (%)				
<2.5	205	82	98	98
$\geq 2.5$	45	18	2	2
Total	250	100	100	100
CD4 cell count/ $\mu$ l				
< 200	81	32.4	17	17
$\geq 200$	169	67.6	83	83
Total	250	100	100	100
Direct antiglobulin test				
Negative	248	99.2	100	100
Positive	2	0.8	0	0
Total	250	100	100	100

**Table 4: Association between reticulocyte count and age/sex of subjects with anaemia**

	Reticulocyte count % $<2.5$		Reticulocyte count % $\geq 2.5$		Total
	Number	%	Number	%	
Age (yrs)					
<25	7	77.8	2	22.2	9
25-40	138	80.7	33	19.3	171
41-50	46	83.6	9	16.4	55
>50	14	93.3	1	6.7	15
Total	205		45		250
Sex					
Male	63	88.7	8	11.3	71
Female	142	79.3	37	20.7	179
Total	205		45		250

Chi-square = 1.7091; Df = 3  $p = 0.6349$ ; Chi-square = 0.1182; Df = 1;  $p = 0.0555$

**Table 5: Association between direct antiglobulin test (DAT) and age of HIV-infected subjects with anaemia**

Age (years)	HIV-infected subjects with positive DAT	HIV-infected subjects with negative DAT	Total
<25	0	9	9
25-40	0	171	171
41-50	1	54	55
>50	1	14	15
Total	2	248	250

Chi-square 8.86, Df = 3,  $p = 0.0339$

## DISCUSSION

That none of the subjects met the criteria that define autoimmune haemolytic anaemia (AIHA) is in agreement with earlier studies which reported that AIHA is rarely seen in HIV infection in Africa. This may partly be attributed to the fact that acquired autoimmune disorders are not

common in many parts of Africa and that Africans that develop autoimmune diseases may have hereditary predisposition.<sup>8</sup> This view that was also supported by Volberding *et al.*, who in their study observed that AIHA is not common in patients with HIV infection.<sup>9</sup> It may also partly be due to the suggestion of a presence of anti-erythrocyte autoimmunity without haemolysis in a large number of HIV-infected patients.<sup>10</sup>

The prevalence of positive DAT of 0.6% (2 of 350) found in this study is in contrast with previous studies by De Angelis *et al.*, and Koduri *et al.*, who reported the prevalence of positive DAT in HIV-infected patients ranges between 18 and 43%.<sup>10,11</sup>

The two subjects that screened positive to DAT in our study, had low mean haemoglobin concentration compared with the mean haemoglobin concentration of those that were DAT negative. Although not statistically significant, this observation corroborates the report by Lai *et al.*, who in a study they carried out in Roma, Italy, observed that patients with positive DAT have low average haemoglobin levels when compared to those who are DAT-negative.<sup>12</sup> Our finding also confirmed the observation by De Angelis *et al.*, who reported that DAT-positive patients have lower haemoglobin levels than DAT-negative patients.<sup>13</sup>

It was noticed that the two subjects that screened positive to DAT were females. This agrees with observation by Jacobson *et al.*, who reported that autoimmune conditions are common in females.<sup>14</sup> Furthermore, that these two subjects were above the age of 40 years, and have a low reticulocyte count may be as a result of decreased erythropoiesis that occurs with increasing age. Since the two subjects also had severe immunosuppression (CD4<sup>+</sup> cell count <200 cells/ $\mu$ l), it may also be due to bone marrow hypoplasia seen primarily in patients with advanced stages of HIV infection.<sup>10</sup> However, though the two subjects had severe immunosuppression, we found no statistical significant association between DAT and CD4<sup>+</sup> cell count in this study.

We observed that subjects with reticulocytosis were more in the anaemic subjects than in the non-anaemic subjects. It is possible that the cause of anaemia in some of these patients is haemolysis from other conditions such as malaria which is endemic in our environment. (Beare *et al.*, 2006).<sup>15</sup> It may also be due to infection by human parvovirus B19 which invades erythroid progenitor cells and then replicates extensively, ultimately lysing the infected cell causing severe chronic anaemia.<sup>16</sup>

## CONCLUSIONS

Autoimmune haemolytic anaemia in HIV infection is a rare complication in our geographical location.

## Limitations of the study

For financial and logistics reasons the sample size was not large enough.

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**How to cite this article:** Adewumi AA, Titilope AA, Osamuedemen VA, Vincent OO, Akinsegun AA, Dapus OD, *et al.* Prevalence of HIV-related autoimmune haemolytic anaemia in Lagos, Nigeria. *Niger Med J* 2014;55:63-6.

**Source of Support:** Nil, **Conflict of Interest:** Nil.