



## **Prevalence of Anaemia after Initiation of Antiretroviral Therapy among HIV-infected Patients Attending University of Calabar Teaching Hospital Calabar, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ECA and JOA managed the analyses of the study. Author JOA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To investigating some demographic variables and red cell parameters of infected persons already accessing antiretroviral therapy with a view to identifying sub- groups with higher proportions of anaemia.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** University of Calabar Teaching Hospital Calabar Nigeria, between August 2017 and July 2018.

**Methodology:** Subjects comprised 60 male and female HIV-infected adults attending University of Calabar Teaching Hospital Calabar, and equal number of age and sex-matched control subjects who were HIV sero-negative as at the time of this study. The infected persons were being treated with either Tenofovir+Lamivudine+Efavirenz (TLE) or Lamivudine+Zidovudine+Nevirapine (LZN). A pre-tested structured questionnaire was administered by two trained interviewers which captured

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the bio-data, sociodemographic variables and therapy-related information. Venous blood was collected aseptically by standard phlebotomy into appropriate sample containers for CD4 and red cell counts by automation.

**Results:** The proportion of anaemia occurring between TLE and LZN users was observed to be in the ratio of 2:3. The RBC count, haematocrit and haemoglobin concentration were significantly lower ( $p=.000$ ), while MCV, MCH and RDW were significantly higher ( $p=.000$ ) in subjects infected with HIV compared to the control subjects, Duration of treatment beyond 6 years significantly raised both MCV ( $p=.007$ ) and MCH ( $p=.006$ ) compared to the first 3 years of treatment commencement, while the MCV, MCH and RDW-SD were significantly higher ( $p=.003$ ,  $.014$  and  $.018$  respectively) among LZN users compared to those on TLE.

**Conclusion:** Human immunodeficiency virus infection triggers pathologic mechanisms that culminate into anaemia. While the use of antiretroviral therapy appears to gradually resolve this derangement, the adverse effects of some of the antiretroviral agents contribute to the persistence of anaemia particularly with increasing years of treatment.

*Keywords: Anaemia; HIV infection; antiretroviral therapy; treatment duration.*

## 1. INTRODUCTION

The discovery of HIV infection also brought about investigations into its features and over time, anaemia has been identified as a cardinal manifestation whose persistence and progression are associated with worsening disease state [1-3]. Decrease in red blood cell production, increase in red blood cell destruction and ineffective production of red cells have all been postulated as possible mechanisms by which anaemia occurs in HIV infection [4]. Apart from having this broad overview, studies also seek to identify the prevailing features that infected persons present with, which sometimes differ on the basis of quite a number of demographic issues such as race, gender, age and socio-economic status. Some of the striking observations about anaemia of HIV include the loss of iron balance which has been noted as a significant complication in HIV infection [5,6], while for those on therapy, the occurrence of anaemia has been associated with use of zidovudine.

Meanwhile in developing societies including Nigeria where nutritional deficiencies persist, anaemia on its own is yet to be effectively controlled and different vulnerable groups are being identified particularly women of child-bearing age and children. It may therefore not be surprising that anaemia constitutes a major health challenge for people living with HIV infection in a country like Nigeria [6-8]. The anaemia occurring in HIV infection appears to be ameliorated by antiretroviral therapy, yet significant proportions of anaemic subjects are found among those who have commenced therapy [7-10]. Apart from establishing that

anaemia also occurs among infected persons on therapy, there is a need to appreciate the more vulnerable sub-groups with higher proportions of anaemic subjects. This is in addition to observing the laboratory features of this type of anaemia. The current study therefore investigated certain demographic variables and red cell parameters of infected persons already accessing antiretroviral therapy at University of Calabar Teaching Hospital Calabar, Nigeria.

## 2. MATERIALS AND METHODS

This study was carried out at the University of Calabar Teaching Hospital Calabar, Nigeria. The study subjects constituted 60 male and female HIV-infected adults with equal number of age and sex-matched control subjects who were HIV sero-negative as at the time of this study. Ethical approval was obtained from the University of Calabar Teaching Hospital Medical Ethical Committee, while informed consent was given by each participant. A pre-tested structured questionnaire was administered by two trained interviewers which captured the bio-data, sociodemographic variables and therapy-related information. Venous blood was collected aseptically by standard phlebotomy into ethylene diamine tetra-acetic acid sample containers for further analyses. The CD<sub>4</sub>T-cell count was conducted using Partec cyflow cytometer, while red cell parameters were studied by automation using Sysmex KX-21N from Sysmex Corporation, Japan. Statistical analyses of data (student t-test and one-way analysis of variance) were carried out using SPSS 20.0. A two tailed P-value of  $\leq 0.05$  was considered indicative of a statistically significant difference.

### 3. RESULTS AND DISCUSSION

#### 3.1 Results

The current study observed a pattern among those accessing the facility. The subjects were mainly above 40 years of age, followed closely by those within 30-40 years, while, few persons were below 30 years. The gender distribution revealed more females were involved compared to the males. Moreover, more of married persons and those with appreciable level of literacy accessed the facility (Table 1).

The HAART combinations given to the subjects were noted as Tenofovir+Lamivudine+Efavirenz (TLE) and Lamivudine+Zidovudine+Nevirapine (LZN). A fair distribution of these protocols was considered in checking for proportions of anaemic subject in the study. This study used a haemoglobin cut-off value of 110 g/l (observed mean value of haemoglobin concentration among HIV-infected subjects from previous studies in the locality). The proportion of anaemia occurring between TLE and LZN users (29 and 31 participants respectively) was observed to be in the ratio of 2:3. Additionally, anaemia occurred the most among those in their first 3 years of treatment, dropped between 4-6 years and picked up again after 6 years of treatment (Fig. 1).

The RBC count, haematocrit and haemoglobin concentration were significantly lower, while MCV, MCH and RDW were significantly higher in

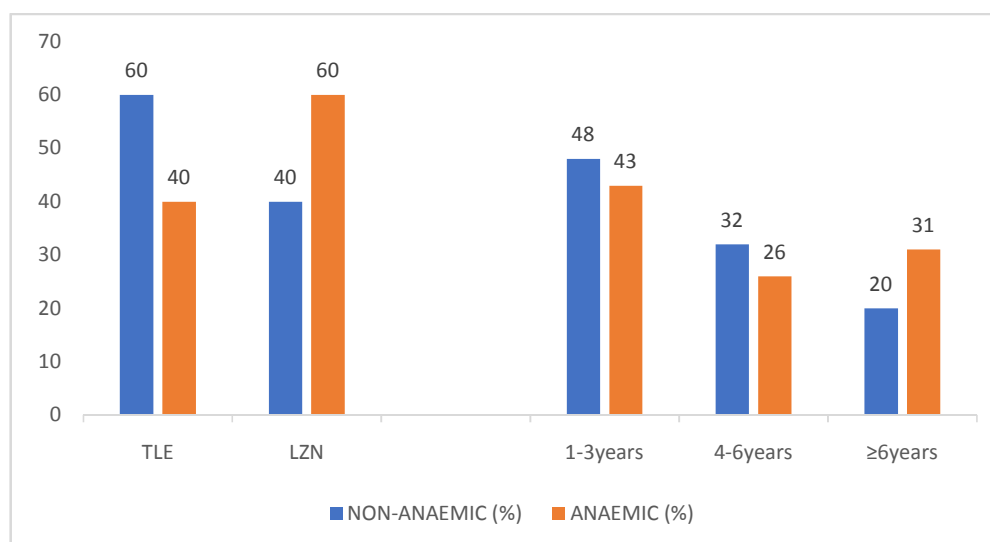
subjects infected with HIV compared to the control subjects (Table 2).

**Table 1. Demographics of the participants**

Demographic variables	Participant distribution % (n)
<b>Age (years)</b>	
<30	20 (12)
30-40	40 (24)
>40	40 (24)
Sub-total	100 (60)
<b>Gender</b>	
Female	62 (37)
Males	38 (23)
Sub-total	100 (60)
<b>Marital status</b>	
Single	30 (18)
Married	63 (38)
Widowed	7 (4)
Sub-total	100 (60)
<b>Educational level</b>	
Primary	17 (10)
Secondary	38 (23)
Tertiary	45 (27)
Sub-total	100 (60)

Duration of treatment beyond 6 years significantly raised both MCV and MCH compared to the first 3 years of treatment commencement (Table 3).

The MCV, MCH and RDW-SD were significantly higher among LZN users compared to those on TLE (Table 4).



**Fig. 1. Proportions of anaemic subjects by drug type and treatment duration**

**Table 2. Red cell parameters of HIV-infected subjects after initiation of HAART**

Parameters	Control Subjects n = 60	HIV Subjects n= 60	p-value
RBC x10 <sup>12</sup> /l	5.49 ± 0.52	4.43 ± 0.58	0.000
HCT l/l	0.41 ± 0.03	0.37 ± 0.03	0.000
Hb g/l	126.55 ± 5.31	108.25 ± 11.02	0.000
MCV fl	71.59 ± 4.74	84.13 ± 9.04	0.000
MCH pg	20.84 ± 1.46	24.27 ± 2.73	0.000
MCHC g/l	291.28 ± 7.73	288.85 ± 7.99	0.093
RDW CV %	14.20 ± 1.05	14.83 ± 1.32	0.005
RDW SD	39.24 ± 3.36	47.75 ± 6.14	0.000

**Table 3. Impact of years of treatment on red cell parameters of the infected subjects**

Parameters	1-3 years n = 27	4-6 years n= 17	>6 years n = 16	p-value
RBC x10 <sup>12</sup> /l	4.54 ± 0.67	4.41 ± 0.42	4.24 ± 0.56	0.265
HCT l/l	0.37 ± 0.04	0.38 ± 0.03	0.37 ± 0.04	0.850
Hb g/l	107.41 ± 12.40	108.35 ± 10.39	109.56 ± 9.64	0.829
MCV fl	80.46 ± 9.95	85.41 ± 6.05	*88.97 ± 7.75	0.007
MCH pg	23.18 ± 2.92	24.54 ± 1.90	*25.84 ± 2.41	0.006
MCHC g/l	288.59 ± 8.48	288.24 ± 7.63	289.94 ± 7.91	0.814
RDW CV %	15.19 ± 1.41	14.51 ± 1.11	14.54 ± 1.30	0.151
RDW SD	46.65 ± 6.79	47.31 ± 5.20	50.08 ± 5.59	0.198

\*significantly higher than those within 1-3 years of treatment

**Table 4. Impact of HAART combination on red cell parameters of the infected subjects**

Parameters	Subjects on TLE n = 29	Subjects on LZN n= 31	p-value
RBC x10 <sup>12</sup> /l	4.55 ± 0.59	4.31 ± 0.56	0.120
HCT l/l	0.37 ± 0.04	0.37 ± 0.03	0.741
Hb g/l	108.45 ± 12.44	108.06 ± 9.72	0.894
MCV fl	80.65 ± 7.64	87.39 ± 9.14	0.003
MCH pg	23.39 ± 2.54	25.10 ± 2.68	0.014
MCHC g/l	289.76 ± 8.25	288.00 ± 7.79	0.399
RDW CV %	14.87 ± 1.41	14.79 ± 1.26	0.836
RDW SD	45.83 ± 4.97	49.55 ± 6.65	0.018

### 3.2 Discussion

Anaemia is one of the common findings among persons living with HIV infection as also reported in studies from Calabar, Nigeria [6,8]. However, the presence of anaemia in HIV infection even after commencement of treatment is an observation that warrants investigation of both demographic and biomedical features of this type of anaemia. This could be of use in recognizing sub-population of infected persons with peculiar needs. The current study observed an important trend in the characteristics of persons accessing antiretroviral therapy at University of Calabar Teaching Hospital, Calabar as at the time of the

study. They were mainly females from 30 years of age with most of them being married and having good level of education. This observation could be because of the maternal health policy for HIV screening during antenatal care [11,12]. Most of the women diagnosed in this manner eventually stay on a life-long treatment course. This finding further reveals that the more responsible persons in terms of age, marital status and educational level are more likely to access treatment implying the need to look out for younger infected persons, single persons as well as educationally disadvantaged persons in our society who are less likely to seek medical attention and care on their own.

Considering the almost equal number of TLE and LZN users enrolled in the study, it is quite interesting to note that the proportion of anaemia occurring between TLE and LZN users was in the ratio of 2:3. The issue of antiretroviral agents being associated with anaemia has been predominantly linked to the use of zidovudine which is reported to have bone marrow suppressing adverse effects. However, the finding of up to 40% proportion of anaemic subjects among the TLE users also call for concern, especially considering that the subjects were relatively stable and had no chronic renal complication as at the time of study. Haemolysis arising from possible development of autoantibodies to the antiretroviral agents has been postulated as a mechanism for anaemia in HIV infection [4] but not much literature exists on specific agents with the adverse effect of haemolysis. While various other factors could be at work, the possibility that the TLE HAART combination in some way may be contributing to early destruction of red cells is worth considering. Along this line of thought, the current observation that in HIV infection, anaemia from marrow suppression as seen among LZN users is higher in proportion compared to that possibly arising from haemolysis (within the TLE group) could be attributed to the fact that haemolysis on its own induces erythropoiesis whereas marrow suppression does not. The study also noted that the proportion of those with anaemia was highest among those in their early years of treatment. (1-3 years), the following three years showed reduced proportion, an improvement attributable to the correction of iron imbalance and dysfunctional erythropoiesis that is high among HAART-naïve subjects. Beyond six years of being on treatment however, the threat of anaemia reemerges, but mainly from long-term exposure to the adverse effects of the antiretroviral agents.

Almost all the red cell parameters of the infected persons undertaking treatment were significantly altered compared to values from control subjects. The red cell count, haematocrit and haemoglobin concentration of those infected were lower compared to the control group. There is anaemia despite being on treatment, with the features of increased cell volume, mean cell haemoglobin and size variability. The finding of macrocytic anaemia in HIV infection is mainly seen among those on HAART, thus reflecting some degree of HAART-associated interference in the normal production of red cells [13-20]. It would therefore

be expected that longer years of therapy may impact more on this derangement. This study observed that duration of treatment beyond 6 years significantly raised both MCV and MCH compared to the first 3 years of treatment commencement, while the MCV, MCH and RDW-SD were significantly higher among LZN users compared to those on TLE. Increase in variation of red cell size in addition to increase also in red cell volume and haemoglobin suggests dyserythropoiesis. This is profound among LZN users and generally for subjects with longer years of treatment (beyond 6years) This observation considered alongside the fact that these sub-groups had high proportions of anaemic subjects, particularly the LZN users, suggest these parameters as possible markers of derangement. Studies with larger sample sizes would be required to confirm this finding. Lately though, antiretroviral therapy has been considered to be contributing to inflammation in HIV infection and increased anisocytosis among those on treatment was reported to align with some markers of cardiovascular risk. This association is thought to be reflective of bone marrow involvement and dyserythropoiesis [21].

#### **4. CONCLUSION**

This study indirectly supports the view that HIV infection triggers pathologic mechanisms such as sequestration of iron, ineffective erythropoiesis and anaemia. While the use of antiretroviral therapy appears to gradually resolve this derangement, the adverse effects of some of the antiretroviral agents see to the persistence of anaemia particularly with increasing years of treatment. Additionally, apart from anaemia being present among the studied population, age, being a female, being married, being educated and having some form of stable income probably play meaningful roles in the accessing of treatment in Calabar, Nigeria.

#### **ETHICAL APPROVAL AND CONSENT**

Ethical approval was obtained from the University of Calabar Teaching Hospital Medical Ethical Committee, while informed consent was given by each participant.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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