Original Research Article

Prevalence of anaemia after initiation of antiretroviral therapy among HIV-infected patients attending University of Calabar Teaching Hospital Calabar, Nigeria

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 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. MATERIAL 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_4T-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 ≤ 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).

Table 1. Demographics of the participants

Demographic variables	Participant distribution % (n)

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

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 (Figure 1).

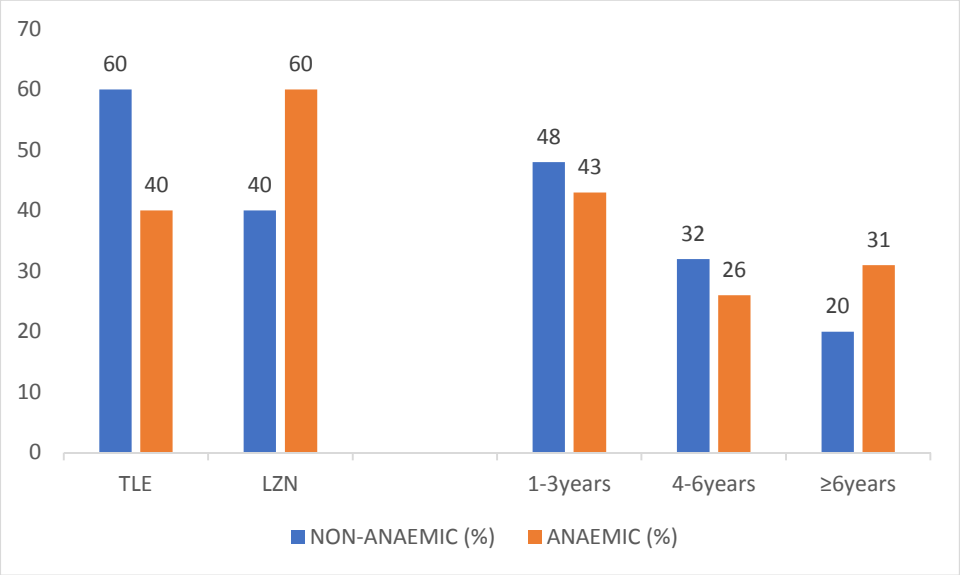

FIG 1. Proportions of anaemic subjects by drug type and treatment duration

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

Parameters	Control Subjects n = 60	HIV Subjects n= 60	p-Value
RBC $x10^{12}$/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

Duration of treatment beyond 6 years significantly raised both MCV and MCH compared to the first 3 years of treatment commencement (Table3).

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 $x10^{12}$/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

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

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 $x10^{12}$/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 remerges, 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.

REFERENCES

1. Moore, RD, keruly LC, Chaisson RE. Anaemia and Survival in HIV infection. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology. 1998; 19 (1) :29-33.

2. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, et al. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. AIDS. 1999;13(8):943–50.

3. Kyeyune R, Saathoff E, Ezeamama AE, Loscher T, Fawzi W, Guwatudde D. Prevalence and correlates of cytopenias in HIV-infected adults initiating highly active antiretroviral therapy in Uganda. BMC Infect Dis. 2014;14:496.

4. Volberding PA, Levine AM, Dieterich D, Mildvan D, Mitsuyasu R, Saag M; Anemia in HIV Working Group. Anemia in HIV infection: clinical impact and evidence-based management strategies. Clin Infect Dis. 2004 ;38(10):1454-63.

5. McDermid JM, van der Loeff MF, Jaye A, Hennig BJ, Bates C, Todd J. et al. Mortality in HIV infection is independently predicted by host iron status and SLC11A1 and HP genotypes, with new evidence of a gene-nutrient interaction. The American Journal of Clinical Nutrition. 2009; 90 (1):225-33

6. Akwiwu EC, Akpotuzor JO, Okon JE, Egharevba O. Iron studies of HIV-infected subjects in Calabar- A Nigerian Perspective. Journal of Medical Science and Clinical Research. 2017; **3**(5): 19572-77.

7. Denue BA, Kida IM, Hammagabdo A, Dayar A, Sahabi MA. Prevalence of anemia and immunological markers in HIV-infected patients on highly Active antiretroviral therapy in Northeastern Nigeria. Infect Dis. 2013; 6:25–33.

8. Okafor AO, Usanga EA, Akwiwu EC. Iron related parameters of HIV-infected patients attending University of Calabar Teaching Hospital, Nigeria. Journal of Dental and Medical Sciences. 2016; 15:65-68.

9. Moore RD. Anemia and human immunodeficiency virus disease in the era of highly active antiretroviral therapy. Semin Hematol. 2000;37(4 Suppl 6):18–23.

10. Semba RD, Shah N, Vlahov D. Improvement of anemia among HIV-infected injection drug users receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2001;26(4):315–19

11. Federal Ministry of Health Nigeria National Guidelines on prevention of mother to child transmission of HIV in Nigeria. Accessed 5 August 2018. Available: https://aidsfree.usaid.gov/sites/default/files/tx_nigeria_pmtct_2010.pdf

12. World Health Organization. PMTCT strategic vision 2010-2015: preventing mother-to-child transmission of HIV to reach the UNGASS and Millennium Development Goals. Accessed 5 August 2018.
Available:https://www.who.int/hiv/pub/mtct/strategic_vision.pdf

13. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL et al. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med 1987; 317 (4):192-97.

14. Snower DP, Weil SC. Changing etiology of macrocytosis. Zidovudine as a frequent causative factor. Am J Clin Pathol 1993; 99 (1):57-60.

15. Eyer-Silva WA, Arabe J, Pinto JF, Morais-De-Sá CA. Macrocytosis in patients on stavudine. Scand J Infect Dis 2001; 33 (3):239-40.

16. Moore RD, Forney D. Anemia in HIV-infected patients receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2002; 29 (1):54-7.

17. Bozzi A, Brisdelli F, D'Alessandro AM, D'Andrea G, Lizzi AR,Rinaldi AC et al. Effects of AZT on cellular iron homeostasis. Biometals 2004; 17 (4):443–50

18. Khawcharoenporn T, Shikuma CM, Williams AE, Chow DC. Lamivudine-associated macrocytosis in HIV-infected patients. Int J STD AIDS 2007; 18 (1):39-40.

19. Kallianpur AR, Wang Q, Jia P, Hulgan T, Zhao Z, Letendre SL et al. Anemia and Red Blood Cell Indices Predict HIV-Associated Neurocognitive Impairment in the Highly Active Antiretroviral Therapy Era.The Journal of Infectious Diseases, 2016; 213(7), 1065–73.

20. Panwar A, Sharma SC, Kumar S, Sharma A. A study of anemia in human immunodeficiency virus patients: Estimating the prevalence, analyzing the causative effect of nutritional deficiencies, and correlating the degree of severity with CD4 cell counts. Med J DY Patil Univ. 2016; 9:312-8.

21. Al-Kindi SG, Zidar DA, McComsey GA, Longenecker CT. Association of anisocytosis with markers of immune activation and exhaustion in treated HIV. Pathology and Immunology. 2017; 2(1): 138-50.