

Original Article

Clinical, Haematological and Immunological Profile of a Cohort of Human Immunodeficiency Virus-infected Subjects Initiating Antiretroviral Therapy at a Tertiary Treatment Centre in Nigeria

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ABSTRACT

Background: Human immunodeficiency virus infection (HIV) continues to be a global health problem. It is a systemic disease that can affect virtually all organ systems including the immunological and haematological systems. Haematological abnormalities are among the most common complications of HIV infection; and are strong independent predictors of morbidity and mortality in HIV-infected individuals. This study aimed to determine the changes in clinical, haematological and immunological profile of HIV-infected patients commencing antiretroviral therapy, as well as the correlation of these parameters with the CD4 cells count.

Method: This study was a hospital-based secondary data analysis from 200 HIV-infected patients aged 18 years and above, who were initiated on HAART and have been on these drugs for 12 months. Relevant data were extracted from patient's case notes. Frequencies, proportions, correlations and paired sample t test were performed and the level of significance was set at p value <0.05.

Results: The mean (\pm SD) age of the studied population was 40.5 \pm 10.9 years and majority were females 68% (n=136). The comparative means (\pm SD) values of the parameters at baseline and 12 months after commencement of HAART were: haemoglobin concentration (10.26 \pm 1.68 vs 11.50 \pm 1.11 g/dl, P value <0.0001), lymphocyte count (40.0 \pm 13.36% vs 44.13 \pm 11.70%, 95%, P value 0.001), eosinophils count concentration (13.46 \pm 6.76% vs 11.39 \pm 6.51%, 95%, P value 0.003) and platelets count (211.73 \pm 108.32 vs 255.98 \pm 92.42 X10⁹/L, 95%, P value <0.0001). The mean (\pm SD) change in CD4 count was (162 \pm 96 vs 346 \pm 173 cells/mm³, 95%, P value <0.0001). The mean (\pm SD) of the BMI before and after HAART were (21.22 \pm 4.26 vs 24.85 \pm 4.47 kg/m², 95%, P value <0.0001).

Conclusion: Haematological abnormalities remain a common finding in HIV-infected patients. The role of HAART in improving the clinical, haematological and immunological parameters in HIV-positive treatment-naïve patients was confirmed by significant improvements observed in the abnormal parameters following HAART use.

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INTRODUCTION

Human immunodeficiency virus infection is a systemic disease that can affect virtually all organ systems including the haematological system. Haematological abnormalities are among the most common complications of HIV infection, affecting all lineages of blood cells with anaemia as the most common observed haematological abnormality.¹ In addition to immunological abnormalities related to HIV disease, haematological abnormalities have been reported as strong independent predictors of morbidity and mortality in HIV-infected subjects.² Anaemia has been found to be associated with CD4⁺ cells depletion and progression to acquired immunodeficiency syndrome (AIDS), as well as one of the predictors of HIV-related mortality and poor response to HAART.^{3,4} HIV-related anaemia is often anaemia of chronic illness which can be caused by many factors. These include cytokine induced bone marrow stem cells suppression from continuous immune activation, premature destruction from decreased pliability of the red cells membrane seen in HIV-infected individuals, and drug related like zidovudine containing regimen and co-trimoxazole.⁵⁻⁷ Other contributing factors include HIV-associated neoplastic diseases, opportunistic infections and infestations, and by effect of autoantibody against erythropoietin.⁸ Neutropenia is another common haematological abnormality seen in the advanced stages of AIDS, which may be caused or exacerbated by concomitant myelosuppressive drugs.¹¹ Thrombocytopenia in HIV-infected has a prevalence range of 4-40%, and it was found to be associate with all stages of the disease.⁹ It has been shown to be associated with an increased morbidity and mortality of HIV patients due to risks of bleeding.⁹ HIV-related thrombocytopenia may results from immune-mediated destruction of platelets, adverse effects of HAART, and bone marrow suppression.⁹ The low platelet count seen in HIV -infection has been associated with increased viral load and predicted a rapid decline of CD4⁺ cells count.⁹ Even though studies have shown that the use of HAART has significantly reduced the prevalence of thrombocytopenia in HIV-infected patients, however low platelet count is still being reported even in patients receiving HAART.¹⁰⁻¹²

Before CD4⁺ cells count assessment became readily available in the

developing countries, lymphocyte count was earlier used as a surrogate for CD4⁺ cells count to monitor immune restoration to antiretroviral therapy.² The above hematological parameters served as prognostic and monitoring parameters in HIV-infected subjects on therapy. Thus, it is vital to determine the extent of hematological abnormalities in such patients with a view to offer an integrated care and service that will improve the overall patient's quality of life. This study will therefore assess the frequency of occurrence of the various clinical, immunological and haematological abnormalities, as well as changes of these parameters in HIV-infected patients on HAART attending HIV treatment and care centre in south-western Nigeria.

METHODOLOGY

This is a hospital-based secondary data analysis carried out on HIV-positive subjects aged 18 years and older, receiving treatment and care at the virology clinic of a tertiary healthcare centre in Nigeria. Study data was collected between 20th September and 20th November 2019. There is an average of 2000 HIV-infected patients who seek for medical treatment at this centre annually. The HIV clinic receives patients referred as HIV-positive following screening tests with rapid kits within and outside the hospital, and offers voluntary counselling and testing. The sample size for the study was determined using the Cochran's formula. A prevalence of 50% was assumed for the proportion of hematological anomaly among HIV-patients. The sample size was rounded up to 200

Ethical approval with a protocol number ERC/2019/06 was obtained from the medical research ethics committee of Obafemi Awolowo University Teaching Hospitals Complex Ile Ife before commencement of this research. The data collected were de-identified and unique research identification numbers were used instead of patient's names, and patient's records were handled with strict confidentiality.

Study Procedure , Data Collection and Management

We retrospectively reviewed the case folders of 200 HIV-

infected patients aged 18 years and above and have been on HAART for 12 months with evidence of good adherence, and achieved plasma HIV RNA <20 copies/ml, with up to date records of laboratory investigation results. Data were extracted from the case folders of each participant. Information on socio-demographic characteristics (age, gender, education level, occupation status and alcohol/cigarette use), medical history including HIV disease stage, history of tuberculosis and HAART information were extracted. Physical examination findings such as body mass index (BMI) and blood pressure were recorded. Laboratory parameters such as complete blood count and differentials values and CD4⁺ cells counts were recorded at HAART initiation (i.e. baseline) and at 12 months post-HAART initiation as the case may be. Patients not up to 12 months on HAART were excluded from the study as well as those patients not virologically suppressed.

Data extracted from case folders were entered and analysed using SPSS version 20.0 software. Study group characteristics were described using descriptive statistics; means and standard deviations for continuous variables when normally distributed, and frequencies and proportions for categorical variables. Paired sample t-test was performed to compare sample means at baseline and 12 months after commencement of HAART. All tests are considered significant at p-value of <0.05 and 95% confidence interval.

RESULTS

Sociodemographic and clinical characteristics of the studied population

The sociodemographic characteristics of the study population are shown in Table 1. From the 200 studied participants, 68% (n=136) were females and 32% (n=64) were males. The mean (\pm SD) age of the studied population was 40.5 \pm 10.9 years. Majority of the studied participants were young and mid adults aged between 18 and 55 years (93%). Of the 200 participants, 75% were self-employed, 70% were married, 6% smoke cigarette and 24% ingest alcohol. There were significantly more males than females who smoked cigarette and ingested alcohol among the study population. More than half of the studied population [55% (n=110)] were initiated on tenofovir-lamivudine-efavirenz/nevirapine (TDF-3TC-EFV/NVP) based HAART regimen. Positive history of tuberculosis was present in 13% (n=26) of the studied population, which is significantly more in

the male subjects than the females. Advanced stage of the disease at baseline with WHO clinical stage 3 and 4 was observed in 47.5% (n=95) of the total participants which were significantly more in females than in males. Furthermore, 47% (n=94) of the overall participants were underweight at baseline, with a BMI of <18.50 kg/m².

Baseline Haematological and Immunological Parameters

Most of the studied participants, 63.5% (n= 127) had a baseline CD4 cells count of < 200 cells/mm³. The commonest haematological abnormalities observed include eosinophilia in 83.5% (n=183), anaemia in 65.5% (n=131) and leucopenia in 53.5% (n=107). Others include thrombocytopenia in 27.5% (n= 55) and neutropenia in 16% (n=32) of the participants (Table 2).

Comparison of Some Clinical and Haematological Parameters with the CD4 Count of the Participants at Baseline.

The subjects that were underweight were significantly more among those with CD4 cells count of < 200 than in those with higher CD4 cells count. However, no significant correlation was observed between body weight and CD4 cells count. Most of the subjects with CD4 cells count of < 200 are in the mid adults age group, however a significant negative correlation was observed between CD4 cells count and age of the subjects. Anaemia was significantly more in those with lower CD4 cells count of < 200 compare to those with higher CD4 cells count, with a significant positive correlation between haemoglobin concentration and CD4 cells count. Lymphopenia even though observed in low percentage of the participants (4.5%), but was significantly more in those with CD4 cells count of < 200 than those with higher CD4 count, with a significant positive correlation between total lymphocyte count and CD4 cells count.

Comparative Analysis of Some Clinical, Haematological and Immunological Parameters at Baseline and 12 Months after HAART

The comparative mean (\pm SD) values of the parameters at baseline and 12 months after initiation of HAART are shown in Table 3. The haemoglobin concentration and the lymphocyte

counts were significantly increased (P value <0.0001) and (P value 0.001) respectively. Similarly, the platelets count also increased significantly (P value <0.0001). However, the eosinophils count significantly decreased (P value 0.003). Furthermore, there was also a significant increase in both the CD4 count and the BMI of the patients after HAART with (P value <0.0001) and (P value <0.0001) respectively.

Table 1: Sociodemographic and clinical characteristics of the study population

Patients characteristics	Study population Total (%) N= 200	Gender Total (%) N= 200		P value
		Males 64 (32%)	Females 136 (68%)	
Age (years)				0.010
Young adults (18-35)	82 (41%)	17 (26.6%)	65 (47.8%)	
Mid adults (36-55)	104 (52.0%)	40 (62.5%)	64 (47.1%)	
Older adults (> 55)	14 (7.0%)	7 (10.9%)	14 (7.0%)	
Occupation				0.890
Salary employed	32 (16%)	11 (17.2%)	21 (15.4%)	
Self employed	151 (75.5%)	47 (73.4%)	104 (76.5%)	
Unemployed	17 (8.5%)	6 (9.4%)	11 (8.1%)	
Marital status				0.010
Married	140 (70%)	55 (85.9%)	85 (62.5%)	
Single	28 (14%)	7 (10.9%)	21 (15.4%)	
Divorced	8 (4%)	0 (0.0%)	8 (5.9%)	
Widowed	16 (8%)	1 (1.6%)	15 (11.0%)	
Separated	8 (4%)	1 (1.6%)	7 (5.1%)	
Smoking (yes)	12 (6%)	10 (15.6%)	2 (1.5%)	0.000
Alcohol intake (yes)	48 (24%)	39 (60.9%)	9 (6.6%)	0.000
Positive History of Tuberculosis	26 (13%)	14 (21.9%)	12 (8.8%)	0.011
HAART combination				0.200
AZT+3TC+NVP/EFV	86 (43%)	27 (42.2%)	59 (43.4%)	
TDF+3TC+EFV/NVP	110 (55%)	37 (57.8%)	73 (53.7%)	
ABC+3TC+EFV/NVP	4 (2%)		4 (2.9%)	
CD4 count(cells/mm3)				0.670
CD4 <	127 (63.5%)	42 (65.2%)	85 (62.5%)	
CD4 >	73 (36.5%)	22 (34.4%)	51 (37.5%)	
BMI				0.340
Normal (18.50 - 24.99)	64 (32.0%)	17 (26.6%)	47 (34.6%)	
Underweight (<18.50) Overweight (25.00 - 29.99)	94 (47.0%)	36 (56.2%)	58 (42.6%)	
	37 (18.5%)	10 (15.6%)	27 (19.9%)	
Obese (≥ 30.00)	5 (2.5%)	1 (1.6%)	4 (2.9%)	
WHO clinical stage				0.030
1	23 (11.5%)	5 (7.8%)	18 (13.2%)	
2	82 (41.0%)	21 (32.8%)	61 (44.9%)	
3	83 (41.5%)	36 (56.2%)	47 (34.6%)	
4	12 (6.0%)	2 (3.1%)	10 (7.4%)	

*AZT= Zidovudine, 3TC= lamivudine, EFV= Efavirenz, ABC= Abacavir, TDF= Tenofovir, NVP= Nevirapine, BMI= Body mass index, HAART= Highly active antiretroviral therapy

Table 2: Comparison of some clinical and Haematological parameters with the CD4 count of the participants at Basidine.

Parameter	Study population N = 200 (%)	CD4 groups		P value
		<200 N= 127 (63.5%)	>200 N= 73 (36.5%)	
BMI				
Normal (18.50 -24.99)	64 (32.0%)	49 (38.6%)	15 (20.4%)	0.047
Underweight(<18.50)	94 (47.0%)	56 (44.1%)	38 (52.1%)	
Overweight (25.00-29.99)	37 (18.5%)	19 (15.0%)	18 (24.7%)	
Obese (≥30.00)	5 (2.5%)	3 (2.4%)	2 (2.7%)	
AGE GROUPS (years)				
Young adults (18-35)	82 (41%)	39 (30.7%)	43 (58.9%)	<0.001
Mid adults (36-55)	104 (52.0%)	77 (60.6%)	27 (37.0%)	
Older adults (> 55)	14 (7.0%)	11 (3.7%)	3 (4.1%)	
Hb level (g/dl)				
Normal	69 (34.5%)	34 (26.8%)	35 (47.9%)	0.020
Anaemic	131 (65.5%)	93 (73.2%)	38 (52.1%)	
WBC (X10⁹)				
Normal	89 (44.5%)	52 (40.9%)	37 (50.7%)	0.389
Leucopenia	107 (53.5%)	72 (56.7%)	35 (47.9%)	
Leucocytosis	4 (2.0%)	3 (2.4%)	1 (1.4%)	
NEUTROPHILS (%)				
Normal	161 (80.5%)	108 (85.0%)	53 (72.6%)	0.009
Neutropenia	32 (16%)	13 (10.2%)	19 (26.0%)	
Neutrophilia	7 (3.5%)	6 (4.7%)	1 (1.4%)	
TLC (%)				
Normal	72 (36%)	56 (44.1%)	16 (21.9%)	<0.001
Lymphopenia	9 (4.5%)	8 (6.3%)	1 (1.4%)	
Lymphocytosis	119 (59.5%)	63 (49.6%)	56 (76.7%)	
EOSINOPHILS (%)				
Normal	33 (16.5%)	13 (5.3%)	20 (6.2%)	0.519
Eosinophilia	167 (83.5%)	107 (94.7%)	60 (93.8%)	
PLATELETS (X10⁹)				
Normal	136 (68%)	83 (65.4%)	53 (72.6%)	0.561
Thrombocytopenia	55 (27.5%)	38 (29.9%)	17 (23.3%)	
Thrombocytosis	9 (4.5%)	6 (4.7%)	3 (4.1%)	

*TLC= total lymphocytes count, Hb= haemoglobin concentration, BMI= body mass index, WBC=white blood cells count CD4=clusters of differentiation

Table 3: Correlation of some clinical and Haematological parameters with the CD4 count of the participants at Baseline.

Parameters	Mean ±SD	CD4 counts (cells/mm ³)		
		Pearson correlation (r)	P value	
Age (years)	40.5±10.9	-0.223	0.001	
BMI (kg/m ²)	21.22 ± 4.26	0.080	0.245	
Hb (g/dl)	10.26 ± 1.68	0.146	0.039	
TLC	40.00 ± 13.36	0.199	0.006	
Neutrophils (%)	47.31 ± 13.33	-0.124	0.088	
Total platelets count (x10 ⁹)	211.73±108.32	-0.046	0.514	
Eosinophils count (%)	13.46 ± 6.79	-0.142	0.059	

Table 4: Comparison of clinical, haematological and immunological parameters of the study participants before and 12 months after initiation of HAART

Parameter	Mean ±SD		Mean of the difference	95% CI		P value
	At Baseline	12 months after initiation of HAART		Lower	Upper	
BMI (kg/m ²)	21.22 ± 4.26	24.85 ± 4.47	-3.63	-3.92	-3.34	<0.001
Hb (g/dl)	10.26 ± 1.68	11.50 ± 1.11	-1.23	-1.41	-1.05	<0.001
WBC (X10 ⁹)	4.75 ± 2.04	4.95 ± 1.68	-0.21	-0.54	0.13	0.222
TLC (%)	40.00 ± 13.36	44.13 ± 11.70	-4.13	-6.45	-1.78	0.001
Neutrophils (%)	47.31 ± 13.33	45.86 ± 11.58	1.45	-0.76	3.67	0.197
Eosinophils (%)	13.46 ± 6.79	11.39 ± 6.51	2.07	0.74	3.41	0.003
Platelets (X10 ⁹)	211.73 ± 108.32	255.98 ± 92.42	-44.24	-58.84	-29.63	<0.001
CD4 (cells/μL)	162 ± 96	346 ± 173	-185	-205.55	-163.45	<0.001

*BMI= body mass index, Hb= Haemoglobin, WBC= white blood cell count, TLC= Total lymphocyte count, PLT=Platelets, CD4= Cluster of differentiation 4

DISCUSSION

Haematological abnormalities are among the most common complications of HIV infection, and these abnormalities affects all lineages of blood cells with anaemia as the most common haematological abnormality seen in HIV-infected persons.¹ These abnormalities have been documented as strong independent predictors of morbidity and mortality in HIV-infected individuals.² From the studied population, majority of the HIV infected subjects were young and mid adults. This group of individuals belongs to the most productive age group in every society, contributing significantly to the economic growth of that society, but perhaps are significantly impaired by HIV infection. This is similar to findings from other studies in Nigeria.^{13,14} This study observed the rate of HIV-infection to be two times more among females than males. The high rate in females may be related to the higher prevalence of HIV-infection among females in sub-Saharan Africa, with females accounting for over 60% of HIV-infected cases.¹⁵ Furthermore, females are more frequently subjected to routine counselling and testing during antenatal services, thus, more cases of HIV-infections are more likely to be detected in females than in males. This is similar to findings by other local studies.^{2,17} We found almost half of the total studied participants to be underweight at baseline, this finding was similar to other studies that reported similar BMI for HIV-infected subjects before commencement of HAART.^{1,18,19} The significant change in the BMI of the studied population observed twelve months after commencement of HAART is most probably as a result of effects of HAART in improving the overall clinical outcome of HIV-infected subjects similar to reports by previous studies.²⁰⁻²² This is contrary to other studies that found no association between HAART use and weight.^{23,24} The significant change in the BMI following HAART initiation has further reaffirmed the role of HAART in improving HIV related morbidity and mortality. Weight loss in HIV infection may be related to several factors such as metabolic alterations, anorexia, malabsorptive disorders, hypogonadism, and excessive cytokines production.²⁵ Studies have reported a significant relationship between weight loss and mortality, disease progression, or both, before the advent of effective antiretroviral therapy (ART) and even in regions where ART is readily available.²⁶⁻²⁸

Anaemia was also another common haematological abnormality

detected in up to 65.5% of the studied population. The high prevalence of anaemia reported from this study may be attributed to late presentation of patients, poor nutritional status (with up to 47% been underweight at baseline), advanced immunosuppression in up to 63.5% and high rate of infection and infestations in sub-Saharan Africa. This is similar to findings from other studies within and outside Nigeria.^{1,2,14,29} The rate is however higher than the low prevalence of anaemia among HIV-infected subjects reported from other studies.^{18,20,30,31} The significant change in the mean Hb observed 12 months after initiation of HAART in the studied population is in keeping with the fact that anaemia is an independent risk factor for decreased survival among HIV-infected persons, and use of HAART for as little as 6 months is associated with resolution of anaemia.³² We did not observe any significant association between the presence of anaemia at 12 months after HAART and the type of HAART regimen used by the studied population. This may be related to the fact that most of the studied participants were on tenofovir-lamivudine-efavirenz/nevirapine regimen.

The observed leucopenia in more than half of the study participants with a normal mean white blood cells count may be related to the different clinical stages of the disease in the studied population. Leucopenia was not found to be significantly higher in the low CD4 group than in the high CD4 group. The fact that - about two-thirds of the participants had significant CD4 cells depletion < 200, it is expected that they have lymphopenia more than lymphocytosis, but the observed level of lymphocytosis may reflect the lack of precision in using lymphocytes count as a surrogate of CD4 cells count as earlier used in the past. The lymphocytosis observed may be a result of abnormal production of inflammatory cytokines as response to viral infections.² Furthermore, the fact that CD4 cells are only a subset of the total lymphocytes, other CD cells may be contributing to the lymphocytosis recorded in our study.² This is similar to findings in a study from Zaria north-western Nigeria by Kusfa et al.² It is contrary to findings by other studies that reported normal to low lymphocytes count in HIV-infected persons.^{1,18,34}

Neutropenia was observed in only 16.5% (n=33) of the total studied population. This is quite low compared to other studies that have reported higher prevalence of neutropenia among HIV-infected subjects within the African sub-continent.^{2,18,33,35} The

aetiology of HIV-related leucopenia and neutropenia is multifactorial involving a direct bone marrow suppression by HIV-infection, effect of myelosuppressive drugs such as zidovudine and cotrimoxazole, and other opportunistic infections like cytomegalovirus, tuberculosis, histoplasmosis and leishmaniasis.^{36,37}

Eosinophilia was the commonest haematological abnormality observed in this study, present in majority of the participants. This high prevalence might be due to fungal infections and parasitic infestations that are quite common in developing countries of sub-Saharan Africa. The fact that most of the patient presented with advanced stage of the disease in this part of the world, thus opportunistic infections and infestations as well as other HIV-related skin rash like eosinophilic folliculitis, popular pruritic eruptions are commonly encountered. A high prevalence of eosinophilia among Cameroonian HIV-infected patients was reported by Ako et al.³⁸ However, much lower prevalence for eosinophilia of 9.7% and 1.6% were reported by Al Mohajer et al.³⁹ and Mathew et al,⁴⁰ respectively in their studies. The significant reduction in the eosinophilia noticed 12 months after HAART may be attributed to the effect of HAART on immune reconstitution and reduced rate of infections and infestations. Even though, the mean value may still appear high, this may be related to drug-induced eosinophilia caused by certain classes of antiretroviral medications like Nevirapine.

Normal platelet counts were observed in most of our patient in this study. However, thrombocytopenia has been shown by many studies to be a common haematological abnormality related to HIV infection in sub-Saharan Africa.¹⁸ This high percentage of patients with normal platelets observed in this study may be attributed to different stages of HIV infection in the studied population. This was similar to other studies in Nigeria.^{2,14} This is quite high compared to low prevalence reported by similar studies.^{1,18,41} The observed significant increase in platelets count after commencement of HAART buttresses the role of HAART in reducing morbidity and mortality related to thrombocytopenia. However, there was no direct correlation between CD4+ lymphocyte counts and platelet counts of the patients at baseline. This finding is in agreement with similar studies.^{1,18} The thrombocytosis observed in a small percentage of the studied population may be related to anaemia observed in some of the participants.

Most of the studied participants had severe immunosuppression at

baseline similar as found in other studies.^{2,31,36} However there is a significant increase in the mean CD4 cells count 12 months after commencement of HAART. A positive correlation was also observed between CD4 cells count and age, total lymphocyte counts and haemoglobin level. This is similar to findings from other studies.^{1,19}

CONCLUSION

Haematological abnormalities remain a common finding in HIV-infected patients. The predominant haematological and immunological abnormalities observed from this study were eosinophilia, anaemia, leucopenia, and profound immunosuppression. HIV-related weight loss remained a problem in our patients due to late presentation and poor access to HAART. However, the significant improvement of these parameters observed 12 months after commencement of HAART has buttressed the role of HAART in improving the clinical, haematological and immunological parameters in HIV-infected patients. Thus, clinical and haematological parameters are important monitoring tools for assessing treatment response and prognosis in HIV-infected patients, in addition to routine CD4 cells count and viral load monitoring.

Conflict Of Interest

The authors hereby declare no conflict of interest in the above study

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