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Assessments of viral load and haematological parameters in HIV patients on HAART in Ilorin, Kwara state

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Abstract

Human immune deficiency virus (HIV) infection represents the most common cause of acquired immune deficiency and leads to clinical disease referred to as Acquired Immune Deficiency Syndrome (AIDS). Highly active antiretroviral therapies (HAART) are the gold standard for the management of HIV disease. Viral load indicates that treatment is effective. A high viral load in a person on treatment indicates either that the medication is not being taken properly or that the virus is becoming resistant to the medication. This is a comparative study aimed at assessing the Viral load count and haematological parameters. 120 HIV positive individual and 40 HIV negative subjects were recruited for this study, while haematological parameters were carried out using Sysmex KX-21 analyser. Results was analyzed using SPSS version 20. Our result shows the following mean absolute counts of haematological parameters; lymphocyte count of HIV positive persons to be 1.42 ± 1.82 , Neutrophil 1.64 ± 2.80 , Monocyte 0.07 ± 0.08 , Basophil 0.05 ± 0.06 Eosinophil 1.71 ± 3.42 while the mean value of TWBC 5.130 ± 1.43 , RBC 3.97 ± 0.48 , HB 11.41 ± 1.65 , MCV 87.054 ± 15.59 and MCHC 0.78 ± 0.08 , 1.10 ± 0.32 , 0.06 ± 0.02 , 0.01 ± 0.01 and 1.14 ± 3.42 respectively among the HIV negative individual. In conclusion, we found that the use of antiretroviral drugs could positively or negatively affect haematological parameters, depending on the choice of combination used.

Keyword: Viral Load; HAART; HIV; Full Blood Count

1. Introduction

Human immune deficiency virus (HIV) infection represents the most common cause of acquired immune deficiency and leads to clinical disease referred to as Acquired Immune Deficiency Syndrome (AIDS) (Tracey, 2017). It belongs to the cytopathic retroviruses group and is known to induce damage or death to body cells. HIV-1 infects humans and chimpanzees with the development of AIDS only in humans. The majority of the infections worldwide involve HIV-1. HIV-2 was initially isolated in Senegal and is known to be endemic in many West African countries, but rarer in other continent (Cheesborough, 2010). HIV is a global health challenge with over 40 million people affected and a major cause of mortality worldwide (Kumar *et al.*, 2006). The prevalence of HIV infection in Nigeria according to the technical report from the department of Public Health National AIDS/STI control programme is about 4.1% (A N C H I V, Nigeria, 2010). The 2009 estimate of people living with HIV in sub-saharan Africa is 22.5 million. This represents about 70% of the global HIV burden with a mortality rate of 2.5 million deaths per annum (Tagoe and Asantewaa, 2011). (e.g tuberculosis) or lymphoma (Hoffbrand *et al.*, 2006; Tagoe and Asantewaa, 2011). HIV infects the CD4 cells and in

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absence of suitable therapy leads to insightful CD4 cell lymphopenia. The progressive decline in CD4 cells eventually leads to the development of opportunistic infections, wasting, cancers and death (Tracey, 2017).

Acquired Immune Deficiency Syndrome is caused by HIV and is characterized by progressive damage to the body immune's system which results in a number of opportunistic infections, immunological and haematological complications (Okolie *et al.*, 2003). Haematological abnormalities are the most common in HIV and these include all extractions of blood cells. (Kirchhoff and Silversti, 2008) CD4 T lymphocytes serve as a marker for immune status. Marked immune compromise, as evidenced by a low CD4 lymphocyte count, is less frequently encountered among individuals with access to HAART, because most guidelines recommend treatment of individuals with CD4 lymphocyte count below 200 cells/mm³ and consideration for treatment if the CD4 count is less than 350 cells/mm³. (Valcours *et al.*, 2006) According to World Health Organization (WHO, 2011) guidelines, preventive therapy should be started when an HIV positive patient who has no symptoms registers a CD4 count under 200 cells per cubic millimeter of blood. Haematological complications among HIV patients are mostly marked cytopenias such as anaemia, neutropenia, lymphopenia and thrombocytopenia (Cobson 2007; Moyle 2002). The incidence and severity of cytopenia generally correlate with the stage of the disease with anaemia being the most commonly encountered haematological abnormality and a noteworthy predictor of progression to AIDS (Volberding 2003; Idigbe 2005). Anaemia and neutropenia are generally instigated by insufficient production of blood cells (because of suppression of the bone marrow by the HIV infection through abnormal cytokine expression and alteration of the bone marrow microenvironment) (Coyle, 1997). Thrombocytopenia is caused by immune-mediated destruction of the platelets, in addition to inadequate platelet production. The incidence and severity of HIV infection is accompanied by marked haematologic changes that complicate health and treatment. There are various peripheral blood and bone marrow changes that are commonly associated with the disease. This is due to its diverse influences on the haemopoietic tissues (Tague and Asantewaa, 2011). The occurrence of thrombocytopenia may be due to immune reaction or effect of antiretroviral drugs or as part of pancytopenia often seen in ill patients with opportunistic infections. A presumptive diagnosis of immune thrombocytopenia may be made in HIV patients without any treatment history or splenomegaly and with bone marrow film studies showing adequate level of megakaryocytes (Costello, 2008). Moreover, the presence of the lupus anticoagulant and anticardiolipin antibodies in patients with HIV infection may play a significant role in HIV-associated thrombocytopenia (Cohen *et al.*, 2008).

Highly active antiretroviral therapies (HAART) are the gold standard for the management of HIV disease (Odunukwe *et al.*, 2005). They were introduced in 1996 and the commonly used HAART regimens include: abacavir, tenofovir, ritonavir, atazanavir, efavirenz, emtricitabine, lamivudine, nevirapine, zidovudine, etc. These drugs have significantly modified the course of HIV disease with longer survival rates and overall improvement of life quality and expectancy in HIV-infected people (Jacobson *et al.*, 2004). However, early data have raised concerns about HAART being associated with an increase in peripheral and coronary arterial diseases, neurotoxicity, osteoporosis, etc (Klein *et al.*, 2005).

Viral load indicates that treatment is effective. A high viral load in a person on treatment indicates either that the medication is not being taken properly or that the virus is becoming resistant to the medication (Jacobson *et al.*, 2004). HIV patients with high viral loads are more infectious and lead to higher HIV transmission rates (Jacobson *et al.*, 2004).

2. Material and methods

2.1. Study area

This study shall be conducted at HAART Clinic located in Ilorin, Kwara state. The study shall be carried out in Ilorin, Kwara state, Nigeria. Ilorin was founded in the late 18th century by the Yoruba people, one of the three largest ethnic groups in Nigeria, in 1450. It became the provincial military headquarters of the ancient Oyo empire in 1817.

The core Ilorin area, about 765 square kilometers, Ilorin is the state capital of Kwara in western Nigeria and has three local Government Areas which are: Ilorin East, Ilorin South and Ilorin West. It is located at 8°30'N and 4°33'E. The average daily temperature is about 27 °C with 81% humidity. It has seasonal variation, with a dry season from December to March and a wet season from June to October. In 2006 a Nigerian census indicated that Ilorin had a population of 777,667 making it the 7th largest city by population in Nigeria and estimated 908,490 in 2011.

It is an industrial, commercial, and educational centre. It is a major market for locally raised crops which includes: yams, cassava, corn, sorghum, millet, rice, peppers, peanuts, shea nuts, cotton. And for cattle, hides and poultry. Local handicrafts which includes: pottery making, wood carving, leather working, cloth weaving, and mat and basket weaving.

The urban economy is dominated by government in the formal sector and trade in the informal one. It has a growing industrial sector includes: soft-drink bottling, sugar refining, match and soap manufacturing and iron working with several banks and insurance companies and several educational institutions.(The world Gazetteer-Ilorin, Nigeria Archived from the original (2013).

2.2. Study design

This is a comparative study.

2.3. Sample size

The sample size will be obtained using the formular of Nianget *al.*,(2008) and stated thus:

$$N = Z^2 \times P (1 - P) / d^2 \text{ (Nainget } al., 2008)$$

N = Minimum sample size

P = Prevalence of HIV in Nigeria = 0.087 (8.7%) (Awofola, 2018).

d = Desired level of significance = 0.05 (5%)

Z = Confidence interval = 1.96 (95% confidence interval)

$$N = (1.96)^2 \times 0.087 (1 - 0.087) / (0.05)^2$$

$$N = 3.8416 \times 0.079431 / 0.0025$$

$$N = 122.$$

Minimum sample to be collected is 122

40 HIV Negative samples shall be employed for this study

Hence, a group of 160 study subjects will be selected for this study.

2.4. Ethical consideration

Ethics approval was sought and obtained from the Kwara State Ministry of Health in Ilorin, Kwara State. Informed consent being obtained with reference number MOH/KS/EU/777/547.

2.5. Full blood count estimation

2.5.1. Principle

The Sysmex KX-21N is a quantitative automated haematology analyser for invitro diagnostic use for determining haematological parameters .Estimation of the numerical and morphologic findings of the complete blood count are useful in diagnosis of such disease state such as anaemia, leukemias, allergic reactions, lymphoma, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM, and MIXED NEUT. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD.

2.6. Procedure for full blood count autoanalyser

2.6.1. Whole Blood Mode (50 uL sample volume)

Procedure

- Before the start-up of the system, the diluent, lyse, cleaner, waste bottle and tubing system was checked to ensure that all the connections were in place.
- The system was then switched ON.
- The blank was run.
- The EDTA anticoagulated blood was mixed in the mixer for 5 minutes.
- The sample initial was inputted.
- The sample cup was then put under the sampling needle and aspiration key was pressed.
- The system aspirates the sample and the sample cup was removed once the sampling needle has retreated inside the instrument.

- The system begins to analyze the sample and once tested, the test result and histogram was printed out with the aid of a preset printer.

3. Results

3.1. Sociodemographic Characteristics of the Study Participants and Control

A total of 120 participants who are HIV positive and 40 control samples were included in this Study. Most of the study participants (79.16.2%) were between the ages of 16 and 65 years and only [25 (20.8%)] were >65 years old. Majority of the participants [89 (74.2%)] were primary school level individual, while secondary school level were [27 (22.5%)] and only 4 (3.3%) were tertiary educated. Majority of the respondents [111 (92.5%)] were living in rural area while only 9 (7.5%) were living in urban area. Most of our study respondents 75 (62.5%) females and only 45 (37.5%) were male. A significant number of HIV positive participants [40 (33.33%)] were traders, followed by unemployed persons 36 (30.0%)

In the control population consisting of 40 people (HIV negative individual), all the participants belonged to age group 16-65 years. Majority of the control were females (24) and only (16) were males.

Table 1 Sociodemographic of the Study Participants and Control

	Test (n=120)	Control (n=40)
Age		
16-30 years	41 (34.16%)	40 (100%)
31-49 years	33 (27.50%)	
50-64 years	21 (17.50%)	
>65 years	25 (20.83%)	0
Gender		
Male	45 (37.5%)	16 (13.33%)
Female	75 (62.5%)	24 (86.66%)
Occupation		
Traders	40 (33.33%)	21 (52.5%)
Teachers	21 (17.5%)	0
Apprentice	18 (15.0%)	19 (47.5%)
Unemployed	36 (30.0%)	0
Others	5 (4.16%)	0
Edu. Level		
Primary	89 (74.2%)	0
Secondary	27 (22.5%)	32 (80.0%)
Tertiary	4 (3.3%)	8 (20.0%)
Residences		
Rural	111 (92.5%)	12 (30.0%)
Urban	9 (7.5%)	28 (70.0%)

3.2. Duration of Infection and HAART Treatment

The medical history of the HIV positive subject was employed using the questionnaire provided for the participants. Majority of the study participants who had been infected with HIV for >5 years was found to be 102 (85.0%), while only

18 were infected for between 1 and 5 years. The duration of treatment among the participants was also gathered, our study found participants on HAART between 1 and 5 years to be 18 (15.0%), while those who have been on treatment for >5 years was found to be the highest frequency (102 85.0%).

Table 2 Duration of Infection and HAART Treatment

	Test (n=120)	Control (n=40)
Years of infection		
1-5 Years	18 (15.0%)	0
>5 years	102 (85.0%)	0
Years of treatment		
1-5 Years	48 (40.0%)	0
>5 years	102 (160.0%)	0

3.3. Mean Difference and p-value of Viral Load and Hematological parameters Between HIV positive and Control

Table 3 Mean Difference and p-value of Viral Load and Hematological parameters Between HIV positive and Control

	Test	Control	p-value
	Mean±SD	Mean±SD	
Parameters			
Viral Load	611.417±306.750	0	0.995
Lymphocyte (%)	58.633±14.75	32.61±0.7	0.634
Monocyte (%)	1.931±1.48	1.71±0.5	0.618
Basophil (%)	1.95±1.44	0.46±0.35	0.741
Eosinophil (%)	2.88±1.168	1.93±1.16	0.313
Neutrophil (%)	35.16±13.022	23.76±1.49	0.895
ANC	1.64±2.80	1.10±0.32	0.04*
ALC	1.42±1.82	0.78±0.08	0.01*
AMC	0.07±0.08	0.06±0.02	0.02*
ABC	0.05±0.06	0.01±0.01	0
AEC	1.71±3.42	1.14±3.42	0.18
MCHCf/l	34.0025±24.335	33.13±1.31	0.921
MCVpg	87.054±15.59	90.09±7.51	0.97
HBg/dl	11.41±1.65	21.44±28.81	0.001*
RBC× 10 ⁶ /μl	3.97±0.48	4.75±0.66	0.551
WBC×10 ³ /μl	5.130±1.43	5.78±1.29	0.321

p-value >0.05 not significant; p-value <0.05* Significant; Key: RBC: Red blood cell count; WBC: Total White Blood cell count; HB: Haemoglobin; PCV: Packed cell volume; MCV: Mean Corpuscular volume; MCHC: Mean Corpuscular Haemoglobin Concentration, ANC; Absolute Neutrophil Count, ALC; Absolute Lymphocyte Count, AMC; Absolute Monocyte Count, AEC; Absolute Eosinophil Count, ABC; Absolute Basophil Count

The mean value of viral load and haematological parameters of subjects and control were computed, some of the haematological parameters among the subjects were found to be high while some are found to be low compared to control group. The mean value for the RBC was low (3.97±0.48 compared to (4.75±0.66) of the control group, the mean value for the HB was also found to be low (11.41±1.68) compared to (21.44±28.81) of the control subject, the statistical

p-value was significant with $p=0.01$ (<0.005), the mean value of MCHC of the subject was found to be negligibly increased (34.002 ± 24.33) compared to (33.13 ± 1.31) of the control group, the mean MCV mean was slightly reduced among the subjects (87.054 ± 15.59) compared to 90.09 ± 7.51 of the control group, the mean value for WBC of the subject was found to be low (5.130 ± 1.43) compared to 5.78 ± 1.29 of the control group. The white blood cell differential count mean value of the subject and control were also compared, the mean value of neutrophil for the subject was found to be slightly increased compared to (23.76 ± 1.49) of the control group, the mean value of the lymphocyte was found to be elevated (58.633 ± 14.75) compared to (32.61 ± 0.7) of the control subject, the mean value of the monocyte for the subjects and control were close to each other, (1.931 ± 1.48) and (1.71 ± 0.5) respectively. About eosinophil, the mean value of eosinophil for the subject was elevated (2.88 ± 1.168) compared to (1.93 ± 1.16) of the control subjects.

3.4. Correlation between Viral load and haematological parameters in HIV subjects

The outcome of the correlation between viral load and haematological parameters shows a positive insignificant correlation with p-value >0.05 at 95% level of significant

Table 4 Correlation Between Viral Load and Haematological Parameters in HIV Positive Patients

	r (n=120)	p-value
Parameter		
Lymphocyte (%)	0.123	0.172
Basophil (%)	-0.09	0.323
Eosinophil (%)	0.096	0.290
Neutrophil (%)	-0.12	0.171
Monocyte (%)	-0.02	0.753
RBC $\times 10^6/\mu\text{l}$	-0.007	0.932
WBC $\times 10^3/\mu\text{l}$	-0.08	0.350
HBg/dl	-0.12	0.185
MCHCfl	-0.22	0.883
MCVpg	-0.39	0.675

4. Discussion

Hematological parameters are important monitoring tools for assessing treatment and prognosis in HIV. The use of antiretroviral drugs could positively or negatively affect these parameters, depending on the choice of combination used. The primary objective of the present study was to evaluate the hematological parameters among HIV reactive patients on antiretroviral therapy for at least 6 months and treatment naïve patients and their comparative analysis. Hematological complications involving all the hematopoietic elements are probably the most frequently encountered complications of AIDS (Zon and Groopman, 1988). Many of these complications have been shown to occur with increasing frequency as HIV infection progresses and a variety of mechanisms appear to play a role in their evolution; such as direct and indirect effects of HIV infection, to the myelosuppressive drugs used in opportunistic infections, and of drugs used as a part of antiretroviral therapy (Moses *et al.*, 1998).

Hematological parameters are important monitoring tools for assessing treatment and prognosis in HIV. The primary objective of the present study was to evaluate the hematological parameters among HIV reactive patients on antiretroviral therapy. The secondary objective was to correlate the hematological parameters of all the patients with Viral load count. In the present study, majority of patients were in the age range of 16-65 years. This study found the haemoglobin value of the subjects to be significantly low (11.41 ± 1.65) ($p=0.01$) compared to 21.44 ± 28.81 of the control population, this was in concordance with a study conducted by Denuet *et al.*, (2013) with mean hemoglobin value of $11.89\pm 1.61\text{g/dl}$ in ART experienced patients. It is also in line with the work of Taha *et al.* (2002), who reported a significant decrease in haemoglobin. This study found the mean value of the RBC to be (3.97 ± 0.48), compared to (4.75 ± 0.66) of the control subjects. Mean value of MCV (mean corpuscular volume) in the present study was found to be low ($87.054\pm 15.59\text{fl}$), among the subject compared to ($90.09\pm 7.51\text{fl}$) of the control. Our study agrees with Jasneet *et*

al., (2017) who found MCV mean (89.40 ± 8.34 fl) among HIV positive individuals. This observation was quite similar to study by Parinitha *et al* and Tripathi *et al.*, (2005). The mean value of MCHC was (34.0025 ± 24.335 g/dl), compared to the control population with mean value of (33.13 ± 1.31 g/dl). Our study agrees with Jasneet *et al.*, (2017) who also found MCHC among HIV reactive receiving treatment to be (32.62 ± 3.58 g/dl). These observations are also in concordance with the study by Parinitha *et al* (2005) and that conducted by Tripathi *et al.*, (2005).

In the present study, the total WBC count was found to be normal among the subjects, the mean value of the subjects was found to be (5.130 ± 1.43), compared to 5.78 ± 1.29 of the control population. This finding is similar to that of Patwardhan *et al.*, (2002), who found normal WBC among 75% and in 70.4% HIV positive patients by Parinitha *et al.*, (2005) respectively. The subjects had raised eosinophil with mean value being (2.88 ± 1.168), compared to (1.93 ± 1.16) of the control population. As for monocyte the mean value was found to be (1.931 ± 1.48), compared to (1.71 ± 0.5) of the control group. Basophil mean was also found to be (1.95 ± 1.44), compared to the (0.46 ± 0.35) of control subjects.

The mean neutrophil count increased (35.16 ± 13.022) compared to (23.76 ± 1.49) of the control populations. This findings disagrees with Akele *et al.*, (2017), who found mean neutrophil count to be reduced in the groups treated with some HAART therapy, the result from our findings may be due to the regime of HAART consumed by our subjects, as observed by Akele *et al.*, (2017), that there was increased neutrophil count in the group given Truvada (NVP), Truvada (EFV), and Lanten (EFV). The four are tenofovir based and the observed increases is supported by the findings of Owireduet *al.*, (2011). Regarding the lymphocyte fraction, our result shows an increase (58.633 ± 14.75) among the subjects compared to (32.61 ± 0.7) of the control population. This finding disagrees with Aupibulet *al.*, (2008), who reported that patient on regimen 5 (zidovudine containing HAART), had statistically significant decrease in lymphocyte count and a significant increase in neutrophil count. The disagreement may be due to consumption of different regimen of therapy among our subjects.

This study found the mean value of viral load among the subjects to be (611.417 ± 306.750). This observation agrees with Marko *et al.*, (2021) who also found (681.82) among HIV reactive patient receiving treatment for 6 months. A low viral load indicates that treatment is effective. A high viral load in a person on treatment indicates either that the medication is not being taken properly or that the virus is becoming resistant to the medication (Global HIV and AIDs, 2017). HIV patients with high viral loads are more infectious and lead to higher HIV transmission rates (Blaser *et al.*, 2014). Furthermore, plasma viral load monitoring is an important marker of response to ART, because a decline in viral load suggests that the patient is adherent to the regimen, that the appropriate doses are being administered, and that the virus is susceptible to the drugs in the regimen (AIDSinfo, 2019). Viral load testing could increase in importance as a guide for clinical decisions on when to switch to second-line treatment and on how to optimize the duration of the first-line treatment regimen (Calmy *et al.*, 2007).

The outcome of the correlation between viral load and haematological parameters shows a positive correlation with insignificant p-value >0.05 at 95% level of significant. This agrees with the findings of Markos *et al.*, (2021) who also found a linear correlation between viral load and haematological parameters.

5. Conclusion

It is concluded that hematological parameters are important monitoring tools for assessing treatment and prognosis in HIV. The use of antiretroviral drugs could positively or negatively affect these parameters, depending on the choice of combination used.

Recommendation

Drugs indeed may not have showed too many side effects on the haematological parameters but there are many physiological and chemical side effects some of which may be irreversible reported by other literatures. It however becomes very critical that proper haematological investigations and considerations be made before and during HAART therapy

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethics approval was sought and obtained from the Kwara State Ministry of Health in Ilorin, Kwara State. Informed consent being obtained with reference number MOH/KS/EU/777/547.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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