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Comparative Assessment of Haematological Profile, CD4 Cell and Viral Load Counts of HIV-Positive Clients Attending Aminu Kano Teaching Hospital (AKTH), Kano State, Nigeria

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Abstract

The cluster of differentiation-4 ($CD4^+$) cell counts and HIV RNA concentration are the biomarkers used in treatment monitoring and HIV disease progression. However, this presents unique challenges in resource-limited settings due to limited healthcare infrastructure, financial constraints, and reduced access to diagnostic tools. This study aims to evaluate and compare the haematological parameters of HIV-positive patients and compare them with their $CD4^+$ cell and viral load counts to ascertain their usefulness in monitoring HIV treatment and disease progression. One hundred and ninety-six (196) HIV-positive clients already on antiretroviral therapy, aged 18-75 years old, were enrolled, and their haematological parameters, Viral load, and $CD4^+$ cell counts were evaluated. The data obtained were analyzed using descriptive statistics, one-way ANOVA, and Tukey's multiple comparison analysis. The mean (±SD) age of the study participants was 39.42 ±3.78 years. A comparison of the haematological indices of study participants based on their $CD4^+$ cell counts reveals a significant difference between the mean of participants in stages I and II of HIV disease progression (P-value: 0.023). Also, the mean lymphocyte counts for participants in stages I and II and between those in stages II and III were significantly different (P-value: 0.0017, <0.0001, respectively). However, when participants were grouped based on their Viral load count, a significant difference was only observed for white blood cell (WBC) count between those whose viral load was below 20 copies/ml and those with above 1000 copies/ml (P-value 0.0421). Conclusively, it was observed that there is a relatively significant difference in WBC count, lymphocyte counts, and percentage lymphocyte count based on the participant's $CD4^+$ cell and viral load count. Mild anaemia was also documented. These findings further substantiate the importance of haematological parameters in HIV treatment and disease progression monitoring.

***Keywords**: HIV, CD4 counts, Viral load counts, Haematological profile, White blood cell counts, Lymphocyte.*

INTRODUCTION

It has been over four decades since the first official report of HIV/AIDS infection in 1981 (NIAID, 2022). Even though the World Health Organization and other organisations have been relentlessly working towards eradicating the pandemic, the infection has remained a major public health problem. Since its discovery, the infection has resulted in an estimated 36.7 million people living with the disease globally (GARPR, 2015). If left untreated, HIV infection progresses in phases and worsens over time, eventually destroying the immune system, thereby leading to acquired immunodeficiency syndrome (AIDS). The first two cases of AIDS in Nigeria were diagnosed in the year 1985 and reported in 1986 (Okoroiwu *et al.*, 2022). Since then, fluctuations in the prevalence of HIV infection have been recorded in the country; a 1.8% prevalence was recorded in 1991 when a watch-out survey was conducted for the first time in the country (FMoH, 2013; Awofala & Ogundele, 2018). An increasing pattern of prevalence was recorded in the subsequent years (3.8% in 1993, 4.5% in 1996, 5.4% in 1999) until the year 2001, when a peak was attained (5.8%), and a decline in trends was observed in the following years (Awofala & Ogundele, 2018). According to UNAIDS (2020) Global AIDS Monitoring Country's progress report, Nigeria has an HIV prevalence of 1.3% as of 2018, and it is currently being ranked as the 4th most globally burdened country with People living with HIV infection (PLWHIV).

The most prevalent indicators of advanced HIV infection and acquired immunodeficiency syndrome (AIDS) are haematologic abnormalities (Erhabor *et al.*, 2005), such as anaemia, neutropenia, and thrombocytopenia (De Santis *et al.*, 2011). Despite being attributed to other factors such as long-term illness, drug side effects, opportunistic infections, and inadequate nutrition, as well as side effects of HIV medication, anaemia is the most encountered blood anomaly in HIV patients (Enawgaw *et al.*, 2014). The prevalence of anaemia ranges from 1.3% to 95% (Akinbami *et al.*, 2010; Behler *et al.*, 2005; Belperio & Rhew, 2004; Dikshit *et al.*, 2009; Patwardhan *et al.*, 2002), which increases in severity with disease progression (Dikshit *et al.*, 2009; Volberding, 2002). Thrombocytopenia is the second most common blood anomaly after anaemia, which is found in 3-40% of individuals with HIV infection and could occur at any stage of HIV infection (Enawgaw *et al.*, 2014).

Nonetheless, most of these markers have not been implemented into routine use because of their purported poor correlation with disease progression (De Santis *et al.*, 2011). Hence, CD4 count and HIV RNA concentration are still the biomarkers used in monitoring HIV disease progression (Enawgaw *et al.*, 2014). However, these tests are not readily available in resource-limited settings, especially in developing and under-developed countries. The study was aimed at comparing the haematological profile of HIV-positive clients on antiretroviral therapy with their CD4 cell count and Viral load counts.

MATERIALS AND METHODS

Study Setting and Population

This cross-sectional study was conducted on HIV-positive Clients attending the US President's Emergency Plan for AIDS Relief (PEPFAR) clinic at Aminu Kano Teaching Hospital, Kano State, Nigeria, from June to September 2022. One hundred and ninety-six (196) consented HIV-positive clients who were already on antiretroviral therapy were enrolled in the study. The sample size was calculated using the Open-Epi version 2.1 statistical package at 99% confidence level. Ethical clearance was obtained from the Aminu Kano Teaching Hospital (AKTH) ethical clearance committee.

Exclusion Criteria

Clients excluded from the study include those with ages below 18 years and above 70 years, pregnant women, those on chemotherapy or radiation therapy due to the tumours, and those on other medication for other illnesses such as tuberculosis, heart disease, and diabetes.

Sociodemographic and Clinical Data

The sociodemographic and clinical details of the study participants were collected using a pretested structured questionnaire through interviews and reviews of their medical records.

Laboratory Tests

For the laboratory analysis, an experienced laboratory technologist took ten millilitres (10ml) of blood samples from the study participants. Plasma HIV RNA viral load count was estimated using Roche Amplicor MONITOR according to the manufacturer's instructions. CD4 cell count was determined using an automated Cyflowmetric machine (Cyflow Partec), and haematological parameters were measured using an automated blood analyser (Sysmex KX-21N), as described by Chukwu *et al.* (2016).

Operational Definitions

The CD4 cell grouping of participants was based on the WHO-recommended classification of HIV disease progression. The first category is Stage I (CD4 cell count ≥500 cells/mm^3), the second category (Stage II; 200-499 cells/mm^3), and finally, the third category (Stage III; CD4 count < 200 cells/mm^3). The viral load count groups are Group A (undetectable viral load group; ≤20copies/ml), Group B (low-level viremia; 21-999copies/ml), and Group C (high-level viremia; ≥1000copies/ml).

Participants were also grouped based on their viral load counts; those with a count less than or equal to 20copies virus per millilitre (≤20copies/ml) of blood samples were placed in Group A (undetectable viral particle), Group B (low-level viremia) consist of participants having between twenty-one to nine hundred and ninety-nine copies of virus per millilitre (21-999copies/ml) of blood sample and Group C (high-level viremia), are participants having one thousand or more (≥1000copies/ml) of a blood sample. For the characterisation of anaemia, participants were further grouped into four categories based on their haemoglobin levels. Those with haemoglobin levels of <12g/dl and >13g/dl (females and males, respectively) are considered Non-anaemic (NA). Haemoglobin levels between 10-11.9g/dl and 10-12.9g/dl (females and males, respectively) are considered mildly anaemic (MiA). Moderate anaemia (MoA) is considered as haemoglobin levels between 7.0- 9.9g/dl, and haemoglobin level >7g/dl severe anaemia (SA).

For the characterisation of cytopenia, the study participants were placed into three groups for each of the cytopenia: (i) those with normal cell counts, (ii) those with high cell counts and (iii) those with low cell counts.

For leucopenia, the cell counts normal range is between 4.0-12.0×10^3cells/µl, while for thrombocytopenia, the normal range is between 150-400×10^3cells/µl.

Data Analysis and Interpretation

Sociodemographic and clinical details were expressed in the form of descriptive statistics. The means of the haematological parameters of groups formed for study participants were compared using one-way ANOVA and Tukey's Multiple comparison tests on GraphPad Prism statistical software Version 9. A p-value ≤0.05 was considered statistically significant.

RESULTS

Sociodemographic Information of Study Participants

the sociodemographic details of the study participants are presented in Table 1. The mean age (±SD) of the study participants was 39.42 ±3.78 years, aged from 18 to 65 years, with the majority being female (67%) (Table 1). The age group 28-37 has the highest number of participants. Of all the participants, only 50(25.51%) were rural residents, while a major proportion were married 134 (68.37%). When characterised based on their literacy status, a significant proportion of the study participants, 73(37.24%), were secondary school certificate holders. Almost half of the participants identified as being unemployed (46.43%), who are mainly female participants (i.e. 58.33% of all the female participants). Only three (1.53%) of the participants were alcohol users.

Table 1: Sociodemographic Characteristics of Study Participants

Variables	Category	Frequency	Percentage (%)
Means Age years ± SD			
	All participant	39.42 ±3.78	
	Male	44.27 ±7.55	
	Female	37.07 ±3.89	
Age (years)			
	18-27	20	10.3
	28-37	66	33.6
	38-47	62	31.7
	48-57	36	18.4
	≥ 58	12	6.1
Sex			
	Male	64	33
	Female	132	67
Residency			
	Urban	146	74.49
	Rural	50	25.51
Marital status			
	Single	20	10.20
	Married	134	68.37
	Widowed	26	13.27
	Divorced	16	8.16
Educational level			
	None	31	15.81
	Primary school	50	25.51
	Secondary school	73	37.24
	Tertiary institution	31	15.82
	Qur'anic school	11	5.61
Occupational status			
	Unemployed	91	46.43
	Student	8	4.08
	Employed	42	21.43
	Self-employed	55	28.06
Alcohol consumption			
	Yes	3	1.53
	No	193	98.47

Clinical Characteristics of the Study Participants

The clinical characteristics of study participants are presented in Table 2. When characterised based on the participant's stage of disease, half 95(50.5%) of the participants were in stage I, 81(41.3%) were in stage II, and the remaining 20(8.2%) were in the third stage of the disease. Furthermore, a significant proportion of the participants have been on antiretroviral therapy for more than five years, with a majority, 156(79.60%) on a first-line regimen, while the remaining 40(31.40%) on a second-line regimen. None of the participants is on a third-line regimen.

Table 2: Clinical Characteristics of Study Participants

Variable	Category	Frequency	Percentage (%)
WHO Disease Staging			
	Stage I	95	50.5
	Stage II	81	41.3
	Stage III	20	8.2
Period on ART (Years)			
	0 -5	63	32.2
	6 - 10	85	43.4
	> 10	40	24.5
Regimen			
	First line	156	79.6
	Second line	40	31.4
	Third line	00	00

Haematological Profile of Study Participants

The one-way ANOVA results comparing the mean haematological profiles of the three groups based on CD4 cell counts revealed differences between the groups for only three parameters: White Blood Cell count (WBC), Lymphocyte count (LYMP), and Percentage Lymphocyte (%LYMP), as presented in Table 3.

Tukey's multiple comparison analysis revealed a statistically significant difference in WBC means between only two groups: participants in Stage I had a higher mean (5.29 ± 1.35) compared to those in Stage II (4.74 ± 13.1), with a P-value of 0.023. For the lymphocyte (LYMP) count, significant differences were observed across all three stages. Participants in Stage I had a higher mean (2.54 ± 0.91) compared to those in Stage II (1.97 ± 0.62) and Stage III (1.87 ± 0.70), with P-values of 0.0017 and <0.0001, respectively. Additionally, for percentage lymphocyte count (%LYMP), participants in Stage I (47.41 ± 9.44) had a higher mean than those in Stage II (43.09 ± 11.00), with a P-value of 0.0211.

The one-way ANOVA comparing study participants grouped by viral load showed significant differences only in WBC counts. Post-hoc analysis indicated that participants in Group A had a significantly higher mean (5.09 ± 1.39) compared to those in Group C (4.29 ± 1.42), with a P-value of 0.0421.

Table 3: Comparison of Haematological Parameters of Study Participants based on CD4 Cell Count

Parameters	Stage I (n = 95)	Stage II (n = 81)	Stage III (n = 20)	ANOVA P Values
WBC ($\times 10^3/\mu l$)	5.29 ± 1.35[a]	4.74 ± 13.1[a]	4.65 ± 1.67	0.0155
LYMP ($\times 10^3/\mu l$)	2.54 ± 0.91[b, c]	1.97 ± 0.62[b]	1.87± 0.70[c]	2.01×10^{-6}
MON ($\times 10^3/\mu l$)	0.38 ± 0.15	0.40 ± 0.33	0.35 ± 0.12	0.62
GRA ($\times 10^3/\mu l$)	2.42 ± 0.89	2.40 ± 1.09	2.45 ± 1.35	0.98
%LYM (%)	47.41 ± 9.44[d]	43.09 ± 11.00[d]	42.31 ± 13.95	0.01
RBC ($10^6/\mu l$)	3.70 ± 0.52	3.68 ± 0.85	3.54 ± 0.86	0.64
HGB (g/dl)	11.50 ± 1.36	11.62 ± 2.01	11.45 ± 3.20	0.88
HCT (%)	35.47 ± 4.24	36.15 ± 6.88	33.82 ± 6.96	0.26
MCV (μm^3)	96.06 ± 11.96	98.73 ± 13.07	97.28 ± 11.42	0.36
MCH (pg)	34.07 ± 4.49	31.79 ± 4.19	33.36 ± 10.60	0.15
MCHC (g/dl)	32.49 ± 2.28	32.14 ± 2.41	34.20 ± 9.00	0.07
RDWC (%)	14.20 ± 1.85	14.33 ± 1.69	14.00 ± 1.71	0.73
RDWS (μm^3)	59.55 ± 14.02	61.71 ± 17.45	59.59 ± 13.72	0.63
PLT ($10^3/\mu l$)	254.68 ± 91.39	240.79 ± 79.02	221.85± 96.36	0.25
MPV (μm^3)	8.68 ± 1.09	8.65 ± 0.96	8.78 ± 1.24	0.91

Values with the same superscript are significantly different based on the post hoc analysis.

Table 4: Comparison of Haematological Parameters of Study Participants based on Viral Load Count

Parameters	Group A (n= 147)	Group B (n=29)	Group C (n=20)	ANOVA P Value
WBC ($\times10^3/\mu l$)	5.09 ± 1.39[a]	5.06 ± 1.29	4.29 ± 1.42[a]	0.050
LYMP ($\times10^3/\mu l$)	2.30 ± 0.86	2.13 ± 0.64	1.92 ± 0.79	0.11
MON ($\times10^3/\mu l$)	0.39 ± 0.27	0.39 ± .12	0.35 ± 0.14	0.77
GRA ($\times10^3/\mu l$)	2.44 ± 0.97	2.53 ± 1.35	2.03 ± 0.78	0.18
RBC ($10^6/\mu l$)	3.71 ± 0.62	3.65 ± 0.45	3.50 ± 1.34	0.46
HGB (g/dl)	11.53 ± 1.61	11.43 ± 1.08	11.80 ± 3.84	0.79
HCT (%)	35.71 ± 4.96	35.31 ± 4.08	35.05 ± 11.31	0.86
MCV (μm^3)	97.13 ± 12.53	97.66 ± 12.35	97.97 ± 11.93	0.95
MCH (pg)	33.25 ± 25.36	31.72 ± 4.24	33.57 ± 10.57	0.94
MCHC (g/dl)	32.30 ± 2.38	32.51 ± 2.26	34.10 ± 8.89	0.11
RDWC (%)	14.27 ± 1.75	13.90 ± 1.76	14.39 ± 1.88	0.53
RDWS (μm^3)	60.67 ± 15.82	58.70 ± 13.71	61.34 ± 15.73	0.79
PLT ($10^3/\mu l$)	246.73 ± 82.04	246.03 ± 108.82	236.60 ± 93.45	0.89
MPV (μm^3)	8.68 ± 1.01	8.63 ± 1.06	8.72 ± 1.35	0.95

Values with the same superscript are significantly different from each other based on post hoc analysis.

Mild anaemia was observed across participants in all three stages of HIV infection (Figure 1). On the other hand, thrombocytopenia was observed, although it was less common, as only 23(11.7%) of the participants exhibited such abnormality. While most participants had normal leucocyte counts, however a notable proportion of participants in Stages II and III exhibited lower-than-normal WBC counts (38% and 36%, respectively) (Figure 2).

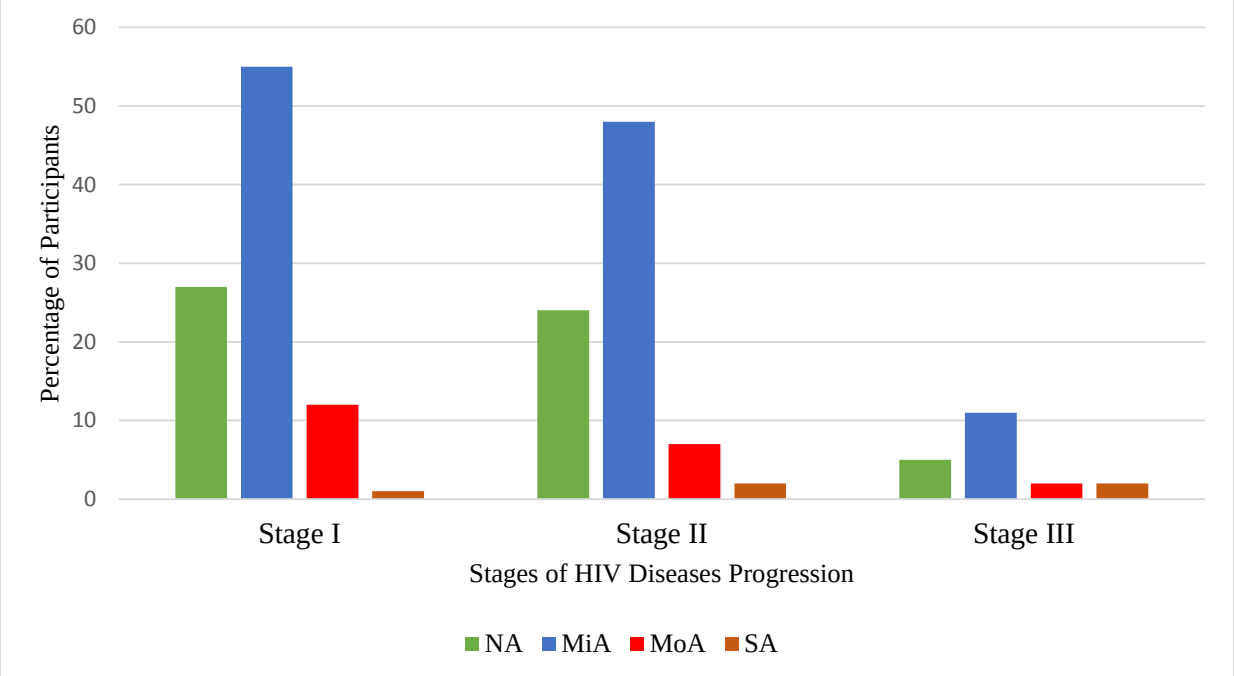

Figure 1: Comparison of Different Grades of Anaemia with CD4 cell count. NA: Non-Anaemic, MiA: Mild Anaemia, MoA: Moderate Anaemia, SA: Severe Anaemia.

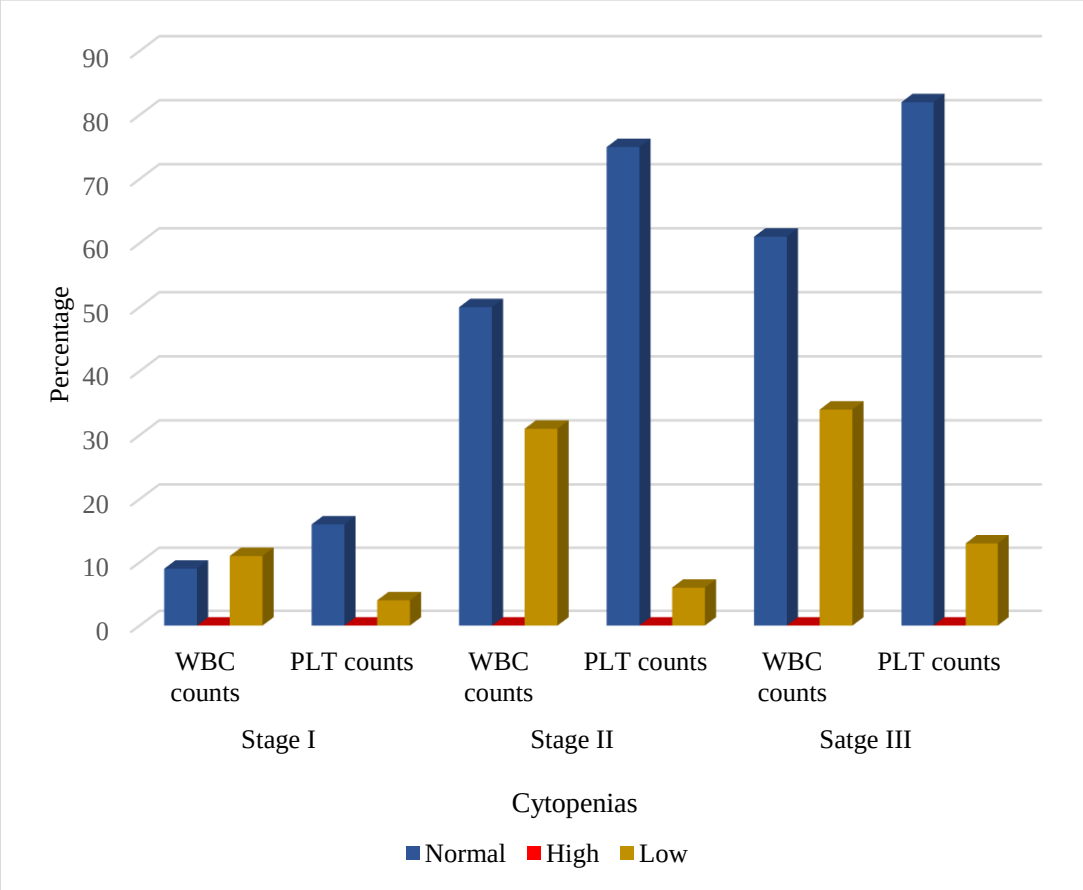

Figure 2: Distribution of Cytopenia among Study Participants based on CD4 cell count

DISCUSSION

The viral load count and CD4 cell count tests are the gold standards used for monitoring HIV disease progression and deciding whether prophylactic treatment against opportunistic pathogens should be started. Nonetheless, the feasibility of these tests is not realistic in resource-constrained environments. Because of this, the WHO recommends using less costly Total Lymphocyte Count (TLC) as a substitute marker in situations where a CD4 count is unavailable or prohibitively expensive (Denue *et al.*, 2013). Furthermore, haematological parameters are frequently impacted in HIV-infected individuals (Sabin *et al.*, 2002), leading to increased morbidity, mortality, and deterioration of quality of life.

This study evaluated the haematological profile of 196 HIV-positive clients in comparison with their CD4 cell and Viral load count. Most of the participants enrolled in this study were female 132(67%), while male participants accounted for only 33%. The high proportion of female participants observed in this study could be attributed to the sampling method used (convenient random sampling). However, according to Global HIV statistics, women and girls represent 53% of all people living with HIV (UNIADs, 2024). Mahathir (1997) reported that women are more susceptible biologically to infection than males due to the larger area of mucous that is exposed to HIV during penile penetration. Moreover, semen can stay in the vagina for several days after sex, further prolonging exposure time to the virus longer in females. Previous studies have also reported a higher prevalence of HIV infection among females than males (Glynn *et al.*, 2001; Girum *et al.*, 2018).

The mean (±SD) age of the study participants was 39.42 ±3.78 years, aged from 18 to 70, with participants in the age group 28-37 having the highest frequency of occurrence. However, this finding might be attributed to one of the criteria used for the enrollment of the study participants (i.e. exclusion of children and adolescents). According to the Nigeria National HIV Surveillance Report (NNHSR, 2024), there is a high prevalence of recent HIV infection among adolescents (15-19 years). Nonetheless, this finding was similar to findings from previous research.

Enawgaw *et al.* (2014) reported a mean (±SD) of 34 ± 9.2 years from a study they conducted in Ethiopia. Afari and Blay (2018) conducted their research on HIV-infected individuals in Ghana, and they reported a mean (±SD) of 41.36 ± 11.36 years. This discrepancy could be due to the differences in the age range (20-70 years) chosen in their study. Another group of researchers (Kusfa *et al.*, 2017) from the Northwestern part of Nigeria reported 35.6 ± 9.65 years, and finally, Damtie *et al.* (2021) obtained 38.8 ± 9.9 years as the mean (±SD) of their study participants.

A majority (50.5%) of the study participants were in the first stage of HIV disease (CD4 cell count ≥500 cells/mm^3), 40.8% were in stage II (CD4 cell count 200-499 cells/mm3), while the remaining 8.2% were in the third stage of the disease, and this could be because the study participants are already taking antiretroviral drugs. This finding was in agreement with that of Seyoum *et al.* (2018), which shows that the majority of their study participants fall under the WHO stage I category. Conversely, the finding of Vansiri and Vadiraja (2016) was contrary to our finding, which reported that only 25% of their study participant had CD4 cell counts <200/mm^3; however, all their study participants were treatment-naive. The finding of Parinitha and Kulkarni (2012) also revealed that only 8.4% of their study subjects were in stage I of the HIV disease progression, though all the study participants were all treatment naïve.

When the haematological parameters of study participants were compared based on their CD4 cell counts, a statistically significant difference was only observed in the mean of WBC of participants in stages II and III (Tukey's multiple comparison tests, P-value 0.023). Furthermore, a significant mean difference was observed for lymphocyte count between study participants in stages I and II (P-values 0.0017) and between the participants in stages II and III (P-values <0.0001). In addition, the means of percentage lymphocyte (%LYM) count of study participants in stages II and III also differs significantly (P-value 0.0211). These findings substantiate the relevance of haematological parameters in HIV disease progression. Though similar research studies have been conducted to validate the usefulness of some haematological parameters as an alternative to CD4 counts in monitoring HIV disease progression and treatment effectiveness, the results obtained have been considerably disparate. However, the findings of Denue *et al.* (2013) and Vanker & Ipp (2014) were similar to those obtained in this study. Similarly, Mbanya *et al.* (2006) also reported a weak correlation.

Meanwhile, for the viral load grouping, a significant difference was observed in only the WBC of participants in stages II and III (P-value 0.023). Generally, higher viral copies will correspond to a low CD4 cell count since the primary target host of HIV is CD4 cells, and CD4 cells are subsets of the WBCs. Thus, this finding is noteworthy since it reveals that study participants with an undetectable level of viral particles have higher WBC than those with high-level viremia.

Another finding in this study is mild anaemia in a significant proportion (58.2%) of the participants. Most of these participants (39 females and 16 males) are in stage III of the CD4 cell count categorisation. This finding also indicates that anaemia is correlated to the severity of HIV disease progression. On the other hand, thrombocytopenia was the only cytopenia observed, and it was less common, as only 23(11.7%) of the participants exhibited such abnormality. The lower occurrence of cytopenia in our study contradicted the findings of another study that compared discrepancies between the haematological parameters of treatment-naïve and experienced HIV-positive clients (Iyabo, 2016). These contradictions are likely because all the participants in this study have been on ART for over a year. Another reason could be the differences in the regimen administered to participants in the two research studies.

CONCLUSION

Conclusively, it was observed that there is a relatively significant difference in white blood cell count, lymphocyte counts, and percentage lymphocyte count based on the participant's CD4 cell and viral load count. Another significant finding in this study was mild anaemia, while thrombocytopenia was less commonly encountered among the study participants. These findings further substantiate the importance of haematological parameters in HIV treatment and disease progression monitoring. These outcomes validate the relevance of earlier researchers' recommendations and findings on the importance of haematological parameters as an alternative tool for HIV disease prognosis and treatment monitoring.

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