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IMMUNOLOGICAL AND VIROLOGICAL MARKERS OF HIV-INFECTED INDIVIDUALS ATTENDING A TEACHING HOSPITAL IN BAYELSA STATE, NIGERIA

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ABSTRACT: Background: Due mostly to the challenge of identifying patients in the early stages of acute HIV infection when HIV serology is still non-reactive, there is a dearth of information regarding the immunological and virological events that take place during this time. Understanding HIV aetiology requires recording immunological and virological markers. This study aimed to analyse the immunological and virological profiles of HIV-infected individuals attending a tertiary hospital in Bayelsa State, Nigeria. Method: A cross-sectional approach was employed, and the study population was drawn from HIV-infected patients undergoing clinical monitoring at the antiretroviral therapy (ART) clinic of the Niger Delta University of Teaching Hospital (NDUTH), Bayelsa State, Nigeria. A whole blood sample was collected from 200 consented subjects: 133 females (66.5%) and 67 males (33.5%). The viral load for the total sample was determined using the Abbott Real-Time HIV-1 Assay US protocol. Results: of the total 200 samples tested, 67 were recorded as undetected, 54 as low levels of virus, 47 as moderate levels of virus, and 32 as high levels of virus. The CD4 counts were carried out using the Partec CyFlow® Counter, with the following results: 49.0% of the sample had above 500 copies of CD4, and 51 of the sample had below 500 copies of CD4. Conclusion: Overall, the information and findings of this study provide fresh insight and knowledge on the CD4 and plasma viral load, and the need for early, individualisation of patient treatment and care and strict adherence to ART dose regimens. Clinicians can leverage these findings to provide more individualistic care to HIV patients for better health outcomes and reduce the rate of transmission in the general population.
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1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) is the final stage of infection with Human Immunodeficiency Virus (HIV). Within the last three decades, HIV/AIDS has killed more than 30 million people worldwide and has been described as the mother of all plagues occurring in the 21st century (Tukura, 2007; Balogun, 2010). After more than forty years, the HIV/AIDs epidemic has remained a public health of international concern despite scientific advances in therapeutic (Hunt et al, 2016) and management, albeit with a reduction in new infections in mostly developed economies. However, the same cannot be said about resource-limited countries of the global south.

HIV-1 subtypes are responsible for the global pandemic of HIV infection (Ndung'ua, and Weiss, 2012; Hemelaar, 2012) and as of 2021, approximately 38.4 million people worldwide were living with HIV (Tee et al., 2022; UNAIDS 2022) HIV-1 shows high variability due to high mutation, recombination, and selective host immune response pressure (Malim, 2009; Wibmer et al., 2013).

As of 2021, approximately 39.9 million people worldwide were living with HIV and it is projected that by the year 2050 about 46 million people will be living with HIV. Of the estimated 39 million living with HIV, about 65 % reside in sub-Saharan Africa, and only half of the new HIV infections were in sub-Saharan Africa (UNAIDS, 2024).

Varied levels of markers of innate and adaptive immune activation remain atypical in many

individuals maintaining durable ART-mediated viral suppression (Hunt et al., 2003; Lederman et al., 2011; Solomon et al., 2003; Wada et al., 2015), and such levels are strong predictors subsequent morbidity and mortality (Kuller et al., 2008).

The pathology of HIV infection is generally characterised by decreasing blood CD4 lymphocyte counts, wasting disease, and neurological disease. The onset of neurological disease is related to infection of macrophages and microglia. During primary HIV infection, only about 50% of infected individuals are symptomatic with fever and lymphadenopathy. After seroconversion, there is an asymptomatic period of 2-15 years whereby viral replication continues at a high rate of up to 1010 infectious virions/day, leading up to approximately 108-109 lymphocytes/day being infected, which are replaced almost as quickly (Tang & Chan, 2001). However, Weiss et al. (2004) reported that the rate of CD4 lymphocyte depletion is not more rapid than observed.

Reduction of HIV-infected lymphocytes occurs through three main mechanisms, namely: (1) direct cytopathic effect (CPE) of HIV, including the formation of syncytia by SI X4 HIV lymphotropic strains, (2) immune destruction of HIV-infected cells by host cytotoxic CD8 T lymphocytes that recognises HIV antigens presented on major histocompatibility complex (MHC) molecules, (3) apoptosis (a process where infected cell commit suicide) due to lymphocyte activation in the presence of specific cytokines (Weiss et al., 2004)

The pathogenesis of HIV involves an intricate interplay of viral replication, host immune response, and disease progression (Oladipo & Awoyelu, 2015). HIV infection demonstrates phases of infection namely the acute, clinical latency, and symptomatic phase. During the acute phase, approximately, 2-6 weeks of HIV post-exposure, there are nonspecific symptoms like fever, fatigue, and malaise which complicate the diagnosis (Alencar et al., 2023). During the clinical latency phase, the virus is active but reproduces at low levels and this phase can last for several years (Alencar et al., 2023). The symptomatic phase is where the infection progresses to the acquired immunodeficiency syndrome (AIDS) stage, which is characterized by severe immune system damage and compromised, and opportunistic infection manifestations (Alencar et al., 2023). HIV-1 strains (CCR5 and CXCR4) influence disease progression, with the R5 tropic virus strain persisting even at AIDS stages, challenging previous assumptions about the role in the late infection stage (Naif, 2013). During the early stage of infection, the host immune response leads to the significant depletion of CD4+ T cells, especially CD4+ cells in the mucosal tissues, before clinical symptoms develop (Lackner et al., 2013).

HIV is a significant public health issue in Nigeria and where the country is classified among the heavy HIV burden counties in sub-Saharan Africa, after South Africa. If concerted effort and research is not undertaken to understand and provide timely updated information on HIV immunological ad virological makers, Nigeria will contribute greater quota to the 2050 projected data of 46 million of person that will live with HIV (UNAIDS, 2024). However, there is a dearth of information regarding the immunological and virological events that occur during this time, the early infection stage of HIV in Bayelsa State, Nigeria, Therefore, this study was undertaken to analyze the immunological and virological profiles of HIV-infected individuals attending a tertiary hospital in Bayelsa State, Nigeria

2. Materials and Methods

2.1. Study Design and Population

This study was cross-sectional in design. The ethical conduct of the work was approved by the Bayelsa State Ethics Committee at NDUTH. The patient's demographic information and past medical records were obtained through the administration of standardised questionnaires. The 200 HIV-1 patients included in this study were all members of the cohort of eligible patients who were HIV-positive. On the other hand, all subjects whose data were incomplete were excluded from the study

2.2 Laboratory Analysis/Clinical Parameters

Viral load and CD4+ cell count were conducted at the University of Port Harcourt's Virus & Genomics Research Unit of the Department of Microbiology

2.2.1 Enumeration of CD4+ Cell

EDTA-treated blood samples were used for CD4 + T cell count using Partec CyFlow® Counter (Partec GmbH, Otto-Hahn-Straße 32, D-48161 Munster, Germany). The CD4 + T cell count was carried out according to the instructions of the Partec CyFlow® Counter manufacturer. The primary outcome of interest was the proportion of HIV-1 individuals who received baseline CD4 +cell count testing within 6 months of diagnosis (Tang et al., 2014).

Data were described and the distribution across four CD4 + cell count categories (<200, 200–349, 350–500, and ≥500) (Tang et al., 2014).

2.2.2. Plasma Viral Load Assay

The Abbott RealTime HIV-1 (m2000sp) assay was used to determine the viral load according to an established protocol (Ribas et al., 2003; Lombart et al., 2005). The results were presented in copies/mL of plasma.

2.3. Data analysis

To evaluate the data, Microsoft Excel version 2021 (Microsoft, USA) was utilised. The statistical significance of every analysis was determined where appropriate using the Chi-square test or Fisher's exact test at a 5% significance threshold.

2.4 Ethical consideration

The study was conducted after obtaining ethical clearance from the University of Port Harcourt Research Ethics Committee.

2. Results

2.1 Analysis of the Study Population

The age range of the 200 HIV-1 positive patients who participated in the study was 5-69 years, with a mean age of 35.3 years. Table 1. indicates by age that the age group 31-40 has the highest number of HIV infections, with 65 cases. This is closely followed by the age group 41-50 with 58 infections. Also, the middle-aged groups—age groups 21-30 and 51-60 show relatively moderate frequencies of 16 and 34 infections, respectively. The youngest age group (0-10) and the elderly group (>60) have lower frequencies of 5 and 9 infections, respectively. Also, the age group 11-20 shows a relatively low frequency of 13 infections.

By gender (Table 1), the data indicate that the number of HIV infections is higher among females (133 cases), representing 67% of the study population, compared to 67 males (33%). This could suggest that females are more vulnerable or exposed to factors that increase the risk of HIV infection in this population (Table 1).

As shown in Table 1, the married category has the highest number of HIV infections, with 101 cases representing 50.5% of the infected study population. Closely followed were single Individuals with 80 cases representing 40% of the infected study population. Furthermore, lower Frequencies in the widowed, separated/divorced, and undisclosed categories were observed as follows 9, 6, and 4 infections, respectively. In terms of educational background, 34.0% of the study population comprised individuals with tertiary education, this was followed by 25.0% of individuals who did not want to disclose their educational status (Table 1).

As shown in Table 1, the unemployed category has the highest number of HIV infections, with 95 cases representing 48.0% of the infected study population. This was followed by students with 37 cases representing 19.0% of the infected study population. Also, civil servants with 31 cases represent 15.0% of the infected study population. Furthermore, lower frequencies in the self-employed, business, traders, farmers, and undisclosed categories were observed as follows 8.0%, 5.0%, 1.0%, and 1.0% infections, respectively (Table 1).

Table 1: Sociodemographic Characteristics of HIV-Infected Participants

Variables	No. Tested (%)
Age Groups (Years)	
0-10	5(2.50)
11-20	13(6.50)
21-30	16(8.00)
31-40	65(32.50)
41-50	58(29.00)
51-60	34(17.00)
Above 60	9 (4.50)
Gender	
Males	67(33.50)
Females	133(66.50)
Marital Status	
Singles	80(40.00)
Married	101(50.50
Separated/Divorced	6(3.00)
Widowed	9(4.50)
Undisclosed	4(2.00)
Education	
None	20(10.00)
Primary	16(8.00)
Secondary	46(23.00)
Tertiary	68(34.00)
Undisclosed	50(25.00)
Occupation	
Students	37(18.50)
Unemployed	95(47.5)
Civil servants	31(15.50)
Trading/Business	27(13.5)
Artisans/Farmer	6(3.00)
Undisclosed	4(2.00)
Total	**200(100.0)**

3.2 Immunological markers of the study population

Figure 1 highlights the immunological and virological markers of study subjects. As described below, for CD4 count, there is a skewed distribution with a significant proportion (34%) of individuals having CD4 counts below 500 cells/mm³, while a substantial number of individuals (68 out of 200) have CD4 counts below 200 cells/mm³, and only 97 individuals (48.5%) have CD4 counts above 500 cells/mm³. The mean CD4 count was obtained as 365.94 cells/mm³.

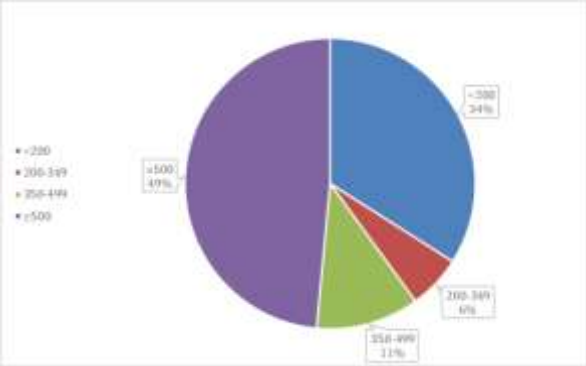

Figure 1. Distribution of Study Participants by CD4 Counts

3.3 Virological markers of the study population

As per the virological markers, the results obtained and as shown in Figure 2, indicate that the category with the highest frequency is undetected (<200 copies/mL) with 67 cases representing 33.5% of the study population that have their viral load suppressed to undetectable levels. Closely followed is the low-level viremia (LLV) (200-500 copies/mL) category with 54 cases, and 47 individuals had moderate-level viremia (501-1000 copies/mL) Further and concerning, 32 individuals have a high- level viremia (>1000 copies/mL) which may be indicative of poor adherence to ART, drug resistance, or late-stage infection and a4s well as coreceptor switch.

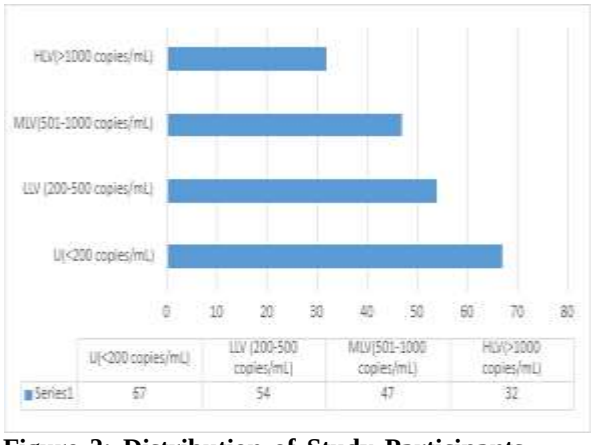

Figure 2: Distribution of Study Participants by Viral Load enumeration
Legend: **U**= undetected; **LLV**= low level of viremia; **MLV**=Moderate level of Viremia; **HLV**= High level of viremia

3. Discussion

This study analysed the immunological and virological profiles of HIV-infected individuals attending a tertiary hospital in Bayelsa State, Nigeria, using the time-dependent variables: CD4 cell count and viral load level.

Immunological and virological markers such as CD+4 cells and viral load are good predictors of HIV morbidity and mortality (Shoko & Chikobvu, 2019), and conversely, a good understanding of these predictors can also mean an elongation of the life of people infected with HIV. Other predictors of HIV infection and establishment of a diseased state in an organism include age and sex. In this study, the median age was 35.5 years, indicating that a lot of the study participants are at their prime, their most productive years, but unfortunately are plagued by this chronic illness-HIV. It was noted that the proportion of individuals infected with HIV increased with increasing age groups, up to 40 – 50 years, after which it decreased as the ages climbed. A large proportion (32.5%) of participants were within the age group, 31 – 40 years, while the least was found within the youngest age group, 0-10 years (2.5%).

Many (67.5%) of the study participants were females, while 33.3% were males. This observation agrees with Ekere et al. (2020) and Okonko et al. (2018, 2020), who also found most HIV-infected subjects to be females. Females are at higher risk of HIV in developing nations, particularly in Sub-Saharan Africa, as evidenced by the larger percentage of females found in the study (Okonko et al., 2020). Also, based on the findings by the NAIIS in 2019 reported that more females (1.9%) than males (0.9%) were living with HIV in Nigeria (NACA, 2019).

Many (50.5%) were married, while 40% were single, and a few (4.5%) were widowed. This is a departure from studies by Tlou (2019), Kposowa (2013), and Shisana et al. (2004), who stated that widowed, divorced and single individuals are at higher danger to contracting HIV infection than their married counterparts.

HIV infection continues to be a public health threat as the infection weakens the immune system, contributing to the advancement of AIDS in the vast majority of infected individuals if left untreated. Therefore, CD4 is an important biomarker that can used to predict the inception of AIDS and track the efficacy of cART (combination of antiretroviral therapy) (Dessie et al., 2020). The average CD4 cell count of the study participants was observed to be 365.7 cells/µl, which indicates a moderate mean CD4 count among the patients. The data show a skewed distribution with a significant proportion (51.5%) of the study participants having CD4 counts below 500 cells/µl. There was a high prevalence of low CD4 counts in 68 out of 200 study participants (34.0%), having CD4 counts below 200 cells/µl, which is the clinical definition of AIDS. This suggests a high prevalence of advanced HIV infection in this population.

Also, about 97 of the study participants (48.5%) have CD4 counts above 500 cells/µl, indicating a lower proportion of individuals with relatively healthy immune systems in the study group. Conversely, the high proportion of individuals with low CD4 (less than 500 cells/µl) counts increases their risk of developing opportunistic infections, which are common complications of AIDS (Benson et al., 2004; Holmes et al., 2006; Lapadula et al., 2015; Root-Bernstein, 2024) The data highlights the urgent need for increased access improvement and adherence to antiretroviral therapy (ART) to improve CD4 counts and reduce the risk of AIDS-related illnesses and death.

This observation partly agrees with Ekere et al. (2020) who also found the fraction of the subjects to increase with increasing CD4 count groups, but the observation was divergent with those with CD4 less than 200 cells/µl being the least with 14%. Okonko et al. (2020) also observed the least percentage (16.8%) of the subjects to have CD counts less than 200 cells/µl. Viral load, also a vital marker in monitoring disease progression as a high level of virus count per ml plasma which inversely means a low level of CD4 count per mm3 shows that important protective cells are being lost by the patient leaving him prone to opportunistic infections (OIs) by other disease-causing agents (Shoko & Chikobvu, 2019). From the current study, majority (74.3%) of participants had lower viral load of <20 – 5000 copies/ml compared to 16.8% of the patients with 5000 copies/ml. This proposes that most of the participants had viral loads that were not so high which validates the higher CD4 counts observed in a greater percentage of these participants. In 8.8% of the participants, the target viral RNA was not detected. Studies have shown that these patients with undetectable viral loads did not transmit HIV to their healthy partners via sexual intercourse (Rodger et al., 2014).

From the current study, the majority (33.5%) of the study participants were in the category with undetectable or very low viral load (<200 copies/mL), indicating that a significant proportion of the population has suppressed viral replication. This is likely due to effective antiretroviral therapy (ART). Also, the frequency of individuals decreases as the viral load increases. This suggests that a majority of individuals in this population are on effective ART, leading to viral suppression. The study also observed that while the number of individuals with high viral loads (MLV and HLV) is lower compared to those with undetectable or low viral loads, it still represents a significant portion of the population that needs to bring down their viral load through improved access and adherence to ART, regular monitoring and treatment to reduce the risk of transmission of HIV by individuals with high viral load.

Studies have shown that patients with undetectable viral loads did not transmit HIV to their healthy seronegative partners through sexual intercourse (Rodger et al., 2014).

5. Conclusion

Overall, the information and findings of this study provide fresh insight and knowledge on the CD4 and plasma viral load as important immunological and virological makers respectively, and good predictors of HIV morbidity and mortality. This gives insight into the proper management of people infected with HIV. Therefore, clinicians can leverage these findings to provide more individualised care to HIV patients for better health outcomes and a reduction in the rate of transmission in the general population.

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Disclosure of conflict of interest

The authors claim that there are no conflicting interests.

Statement of ethical approval

All authors declare that all experiments have been examined and approved by the University of Port Harcourt Research Ethics Committee. Therefore, the study is performed following the ethical standards

Statement of informed consent

All authors declare that informed consent was

obtained from all individual participants included in the study.

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