

# Gender-based differences in hematological and Cd4+ T-lymphocyte counts among HIV-patients in Ido-Ekiti

## Abstract

**Background:** Differences in prevalence and severity of HIV/AIDS between males and females remain complex and controversial; this may vary with time in the same location during the course of the epidemic, and vary greatly by place of residence. The aim of the study was to assess existent sex variations, the efficacy and ability of HAART to resolve hematological and immunological abnormalities in HIV infected.

**Methodology:** A total of 400 HIV infected participants were recruited for the study from Federal Teaching Hospital, Ido-Ekiti between August-2013 to February 2014. 4ml of blood samples were collected into K<sub>2</sub> EDTA bottles for the analysis of hematological and immunological parameters. The volunteers comprised of both male and female respectively 123 (54 on HAART and 69 HAART naïve) 277 (146 on HAART and 131 HAART naïve).

**Results:** Hematological and immunological parameters were found to be significantly different ( $p < 0.05$ ) among male and female patients under HAART.

**Conclusion:** This study revealed that there is significant difference in hematological and immunological parameters between male and female on HAART and HAART naïve patients.

**Keywords:** hematological and immunological parameters, HIV-patients, sex, epidemic, K<sub>2</sub> EDTA

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**Abbreviations:** PCV, packed cell volume; PLT, platelets; WBC, white cell count, LYM, lymphocytes; NEU, neutrophils; MXD, mixed; SD, standard deviation; HAART, highly active antiretroviral therapy

## Introduction

Nigeria is now the second largest HIV disease burden in the world with 3.2 million after South Africa which has 6.8 million burden of the disease though prevalence is stable at 3.4%.<sup>1-3</sup> The first HIV/AIDS sentinel survey in Nigeria was conducted in 1991 with 1.8% prevalence reported. This was followed by 3.8% in 1993, 4.5% in 1996, 5.4% in 1999 and a 5.8% peak in 2001. From 2001 a somewhat decline in trends were noted, starting with 5.0% in 2003, 4.4% in 2005, 4.6% in 2008, 4.1% in 2010 and 3.4% in 2013.<sup>1,2,4</sup> However, the HIV prevalence is much lower in Nigeria when compared to other African countries.<sup>5</sup> Recently, it is estimated that about 3, 229, 757 people live with HIV in Nigeria and about 220, 393 new HIV infections occurred in 2013 and 210,031 died from AIDS related cases. People practicing low-risk sex are the driving force of HIV epidemic in Nigeria while the high risk groups involving female sex workers, and injecting drug users contribute substantially to new infections.<sup>6</sup> The differences in prevalence and severity of HIV/AIDS infection between males and females remain complex and controversial; this may vary with time in the same location during the course of the epidemic, and vary greatly by the place of residence, of all people living with HIV globally, 9% of them live in Nigeria.<sup>7</sup> Gender based differences of HIV/AIDS depend on patterns of disease transmission, as well as on the stage of the epidemic. Approximately half of the people living with HIV

are women. The highest HIV prevalence found among women is in countries where the epidemic has become generalized; since the main transmission pattern is heterosexual, hence women are more exposed to HIV infection. Many women are infected with HIV by their long-time trusted partners or husbands. Women are at a greater physiological risk of contracting HIV than men, because women have a greater mucosal surface area exposed to pathogens and infectious fluid for longer periods during sexual intercourse and are likely to face increased tissue injury. Young women are at particularly high risk due to immaturity of the opening of the womb (cervix), which has not acquired sufficient thickness to act as an effective barrier, thereby facilitating greater exposure of target cells to trauma and pathogens in the vagina.<sup>8</sup>

Women living with HIV have mainly become infected in heterosexual relationships and often in a marriage context. However, Females were often experience the impact of HIV more severely than men, due to a combination of biological, social, cultural and economic factors that contribute to women's vulnerability to HIV. Hematological abnormalities are common complications of human immunodeficiency virus infection, as they increase with the advanced stage of diseases. These abnormalities increase as the disease advances. In both antiretroviral-treated and untreated individuals, different types of hematological abnormalities occur.<sup>9-11</sup> Anemia is the most common hematological abnormality in human immunodeficiency virus (HIV) patients. Several factors play a role in the development of anemia in patients with HIV, including chronic disease, opportunistic infections, nutritional deficiencies and toxicities from medications. As HIV disease progresses, the prevalence and severity of anemia also

increase.<sup>12,13</sup> Thrombocytopenia is another frequent hematological complication of human immunodeficiency virus infection which can occur at any stage of HIV infection. Chronic infection with HIV is now well-characterized causes of chronic immune thrombocytopenic Purpura.<sup>14</sup> The possible mechanisms that have been reported are immune-mediated destruction of platelets by antibodies, cross-reacting antibodies that are directed toward HIV proteins, particularly GP120 and P-24. This type of platelet destruction is called immune thrombocytopenic purpura (ITP) which is characterized by very low platelet counts with normal hematocrit and white blood cell count. Neutropenia is also common leucopenia which occurs in HIV infected individuals. HIV infection suppresses the bone marrow resulting in decreased levels of granulocyte colony-stimulating factor, the factor that stimulates production of white blood cells in the bone marrow and which affects the granulocyte-macrophage lineage, causing in leucopenia and neutropenia. Also, myelo suppressive drugs or the opportunistic infections including cytomegalovirus, tuberculosis, histoplasmosis and leishmaniasis may cause leucopenia. Furthermore, HIV infection can directly result in lymphopenia as the infection progresses, leading to a decrease in CD4+ lymphocytes.<sup>11,15</sup> The CD4+T lymphocyte count is the determination of the concentration of CD4+T lymphocyte in the blood. It is a measure of the immune system which indicates the stage of disease progression in an individual with HIV-infection, a lower count indicating a more advanced stage of the disease.

World Health Organization recommended that most treatment initiation decisions be guided by CD4 measurement and clinical staging.<sup>16</sup> It was reported that, there is a good correlation between CD4 count and development of various complications in HIV/AIDS. It is clear that late starters of highly active antiretroviral therapy with CD4 count <200cells/μl have significantly poor response to therapy and a worse prognosis when compared to early starters with higher CD4+Tcell count.<sup>17-19</sup> General treatment guidelines for the treatment of HIV-infected patients in many countries have adopted three approaches for the initiation of antiretroviral therapy. Early intervention in asymptomatic patients involves the commencement of antiretroviral therapy once the CD4 count is less than <500cells/μl. A less intensive approach is to recommend antiretroviral therapy when the CD4 count falls to 350cells/μl. There is an increasing recognition that poverty in Africa is a critical factor in the transmission of HIV/AIDS, countries where national programs have limited financial resources, treatment decisions are typically delayed until the CD4 count becomes less than 200cells/μl.<sup>20-22</sup> McGrath have reported that time to ART eligibility was significantly shorter for men, underlining the need to develop gender oriented strategies throughout HIV care in the African context, since they reported that men are more likely to present for care with slightly lower CD4 cell counts than women and there is a need to find ways to provide early treatment to male patients.<sup>23</sup> According to UNAIDS, more than 3.9million HIV patients have received antiretroviral therapy (ART) in sub-Saharan Africa at the end of 2009. This represents 37% of those in need of treatment and an increase of one million patients in each year.<sup>24</sup> Early initiation of ART in the course of disease is associated with better survival.<sup>25,26</sup> and better long-term immune reconstitution.<sup>27,28</sup> Introduction of highly active antiretroviral therapy (HAART) in developed countries in the late 90s has resulted in a remarkable decrease in AIDS-related mortality. This decrease in mortality has changed the perspective of HIV infection from that of a rapidly fatal to a chronic manageable infection. Clinical benefits of HAART are due to its effectiveness in

decreasing disease progression in HIV infected patients by sustained suppression of viral replication. The aim of this study was to assess the gender-based differences in some hematological and immunological parameters of medically treated HIV-patients in Ekiti, Nigeria.

## Materials and methods

### Study location

The study site was Ido town, the headquarters of ido-osi local government in Ekiti State, Nigeria. The secretariat sited in between Ido town and Usi town. It is very close to other local government districts, (Moba, Ijero, Ilejemeje and Ado). The local government comprises rural towns: Aaye, Ido, Usi, Ayetoro, Ilogbo, Osi, Ifaki, Orin, Ora, Igbole and some other smaller villages, inhabited mainly by the Ekitis, but with some non-Ekitis fund living peacefully among the people. People in Ido-Osi cherish farming, education, trading and practicing majorly Christianity religion. According to 1991 Census, the Local government has a total population of 107,000 people with eleven electoral wards in the Local government. The climate is characterized by two main seasons; the rainy and the dry season, the rainy season starts in March to October while the dry season is between November to February. The total rainfall in the area is 450mm giving a mean monthly rainfall of 121mm. There is a sharp fall in rainfall at a period between July and August. Temperature in the region is high throughout the year with a means monthly temperature of 27°C and a range of 3.7°C between the month of highest temperature (February) and the month of lowest (August).<sup>29</sup>

### Study design

A total of 400 HIV infected patients were recruited as participants in the study Among the 200 medically treated patients, the males were 54 and the females were 146. Likewise, the untreated patients included 69 males and 131 females. Institutional Ethical Committee/clearance/Permissions were obtained from the Federal Teaching Hospital, Ido-Ekiti, to conduct the study. 4ml of blood were collected into dipotassium ethylene di-amine tetra acetic acid (K<sub>2</sub> EDTA) vacutainer bottles for the analysis of hematological and immunological (CD4) parameters. For this cross-sectional investigation, prior -written informed consent was obtained from all the patients of HIV clinic at Federal Teaching Hospital, Ido-Ekiti (Nigeria), during Agust-2013 to February 2014.

### Hematological parameters were analyzed using hematology analyzer (Sysmex automated hematology analyzer model KX-21N, manufactured by sysmex co-operation Kobe, Japan)

Three parts differential hematology analyzer was used which consist of lymphocyte count (LYM), neutrophil count (NEU), sum of eosinophil, basophil and monocyte as mixed (MXD).

### Principle

The aspirated blood sample is measured to a pre determined volume diluted at the specified ratio and then fed into each transducer chamber, which has a minute hole aperture and also contains electrodes through which direct current flows. Blood cells suspended in the diluents sample, pass through the aperture, causing direct current resistance to change between the electrodes, and blood cell size is detected by electric pulses. Blood cell count is calculated by counting the pulses and the histogram determined by the pulse sizes.<sup>29</sup>

## Procedure

Well mixed EDTA blood sample was used for the analysis of complete blood count, blood sample was aspirated through the sample probe one after another by pressing start switch, sample was analyzed, rinsed and the results displayed on the LCD screen of the machine. After the analysis, machine was shut down by aspirating cell clean which washed and rinsed the machine before finally shutdown and switch off from the power source.<sup>29</sup>

**Calibration of Cyflow counter:** Blood samples for CD4 count were prepared and run on the Partec cyflow counter, according to the manufacturer's instructions. Cyflowcounter was first calibrated to ascertain optimal equipment performance by using count check beads of already known concentration following daily cleaning procedure.

**Cyflow counter count check beads calculation:** A known concentration of 23,470cells/μl count check bead was used, absolute CD4 count value of the count check bead of 966cells/μl with pre-set dilution factor of 42, was used to calibrate the equipment.

**Principle and procedure of flow cytometry for CD4 count:** The counter separated the CD4+ T cell from the monocytes-CD4 bearing cells and noise using a gating system. 20μl of well-mixed whole blood sample was added to 20μl of CD4 MAB (Monoclonal Antibody) in a Rhören tube. This was incubated for 15minutes in the dark. 800μl of CD4 no-lyse buffer was added the mixture was analyzed on Parteccyflow counter and the results were recorded in cells/μl.

## Statistical analysis

The obtained results obtained were analyzed using student t-test to compare the means. Analysis was performed using computer database software from the statistical package for social sciences (version 16.0 SPSS). A P-value of <0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

## Results

A total of 400 HIV infected patients who was categorized into two, 200 HAART naïve treatment and 200 on HAART treatments were involved in this study. The mean±SD of CD4, WBC, PCV, PLT, LYM, NEU and MXD were 152.72±177.22, 4.08±2.63, 32.24±6.29, 164.29±50.38, 37.06±14.57, 51.32±15.49 and 11.18±7.37 respectively in patients on HAART naïve patients and 543.73±295.79, 5.08±1.85, 35.37±5.35, 251.91±73.87, 46.73±12.25, 41.53±12.87 and 11.85±8.48 respectively for HAART patients. All the parameters analyzed were statistical significantly higher in HAART patients except in NEU when compared to the HAART naïve patients, as shown in Table 1. Out of 400 HIV infected patients that participated in this study, 277 were females (146 on HAART and 131 HAART naïve) and 123 were males (54 on HAART and 69 HAART naïve). Mean±SD of CD4, WBC, PCV, PLT, LYM, and MXD were statistically higher in males when compared to the females except NEU in HAART naïve patients. However, in HAART patients, mean±SD of CD4, WBC, PLT, LYM, and MXD values were higher at statistically significant in female compared to male except PCV and NEU as shown in Table 2 & 3.

**Table 1** CD4 counts and hematological parameters of HIV positive patients (HAART & HAART naïve)

Parameters	Mean±SD	t-value (significant)	Mean±SD	t-value (significant)
CD4	152.72±177.22	12.19(0.00)	543.73±295.79	25.99 (0.00)
WBC	4.08±2.63	25.86(0.00)	5.08±1.85	38.77 (0.00)
PCV	32.24±6.29	72.47(0.00)	35.37±5.35	93.54 (0.00)
PLT	164.29±150.38	15.45(0.00)	251.91±73.87	48.23 (0.00)
LYM	37.06±14.57	35.98(0.00)	46.73±12.25	53.99 (0.00)
NUE	51.32±15.49	46.84(0.00)	41.53±12.87	45.65 (0.00)
MXD	1.18±7.37	21.06(0.00)	11.85±8.48	19.25 (0.00)

**Table 2** Sex-wise differences in CD4 counts and hematological parameters of HIV positive patients on HAART Naïve

Sex	CD4	WBC	PCV	PLT	LYM	NEU	MXD
Male (N=69)	163.16±257.01	5.13±3.38	33.81±6.86	176.07±205.47	38.98±13.46	48.82±13.91	11.99±9.98
Female (N=131)	147.23±115.14	4.63±2.12	31.4±5.83	158.09±111.46	36.05±15.07	52.64±16.16	10.75±5.51
F (p-value)	0.87 (0.35)	1.38(0.24)	1.55(0.22)	2.98(0.08)	1.23(0.27)	3.07(0.08)	1.32(0.25)

**Table 3** Sex wise difference in CD4 counts and some hematological parameters of HIV positive patients on HAART

Sex	CD4	WBC	PCV	PLT	LYM	NEU	MXD
Male (N=54)	523.74±314.13	4.94±2.03	37.39±6.35	251.85±69.84	46.99±13.18	41.62±13.28	11.19±4.9
Female (N=146)	689.12±289.49	5.13±1.79	34.62±4.73	263.93±75.54	47.64±11.93	41.49±12.76	12.09±8.83
F (p-value)	4.23(0.02)	0.2(0.65)	6.19(0.01)	0.13(0.72)	1.52(0.22)	0(0.99)	0.92(0.34)

## Discussion

In this present study, female had prevalence of HIV infection with height frequency of HIV Patients on HAART compared to male which

may be due female sexual workers, this confirmed the recent study that the prevalence of HIV infection is higher for females than male.<sup>4,6</sup> Similar to previous studies by Bamlaku et al.,<sup>30</sup> Denué et al.,<sup>31</sup> Yakubu et al.,<sup>32</sup> Ibrahim et al.<sup>33</sup> they reported high prevalence of HIV infection

among female.<sup>31–34</sup> This study supported the fact that Women are at a greater physiological risk of contracting HIV than men because women have a greater mucosal surface area exposed to pathogens and infectious fluid for longer periods during sexual intercourse<sup>8</sup> especially when exposed to unprotected heterosexual intercourse. Hormones such as progesterone are reported to be playing a role in a woman's biological vulnerability to HIV infection.<sup>34</sup> Observational evidence suggests that progesterone containing injectable contraception depot medroxy progesterone acetate (DMPA) may be putting women at higher risk of HIV acquisition.<sup>34–37</sup> Earlier studies showed that pregnant women are at a higher risk of HIV infection than lactating or other women, possibly due to physiological changes that a woman undergoes during pregnancy.<sup>38</sup> High levels of oestrogen and progesterone either during pregnancy or from exogenous sources could cause changes in the structure of the genital mucosa or cause immunological changes, such as an increase in mucosal lymphoid aggregates or hormone-induced over expression of co-receptors associated with HIV infection. Supporting evidence suggests that women have a window of vulnerability approximately seven to ten days after ovulation during their menstrual cycle in which the potential for viral infectivity in the female reproductive tract is increased. This could be due to the suppressing influence of sex hormones on the innate, humoral and cell-mediated immune systems which this takes place in the upper and lower female reproductive tract, and overlaps with the up-regulation of co-receptors for HIV uptake and the recruitment of potentially infectable cells.<sup>39</sup> This present study supported fact that women are tested for HIV earlier than men and had their earlier access to treatment and clinical care compared to men, because of the increasing availability of antenatal testing as part of ongoing expansion in voluntary counseling and testing.<sup>40,41</sup> Early access to treatment and clinical care in female improve their health condition compared to their male counterparts at enrollment into care. In this study, Patients on treatment shows greater improvement in immunological and hematological parameters compared to treatment naïve patients of both sexes, which confirmed the earlier report by Kumarasamy et al.<sup>42</sup> that both men and women showed consistent improvement in the HIV patients on initiating HAART.<sup>42</sup>

## Conclusion

The females have been found to be prone to high risk of HIV infection when compared to their male counterparts; the study indicated that there exist significant differences in the values of hematological and CD4 count features between males and females in relation to HAART and HAART naïve. Hence, encouraging HIV testing among the Nigerian population to ensure everyone knows their HIV status together with efficient linkage to care for newly diagnosed HIV cases is key to mitigate new infections and provide HIV treatment to all.

## Acknowledgements

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## Conflict of interest

The author declares no conflict of interest.

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