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RESEARCH PAPER

# THE EFFECT OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION ON CD4 T-LYMPHOCYTE DEPLETION AMONG PEOPLE LIVING WITH HIV AND AIDS IN ENUGU, NIGERIA.

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## ABSTRACT

This study evaluated the effect of HIV infection on CD4 T-lymphocyte depletion in people living with HIV/AIDS. Ninety (90) subjects recruited into this study were grouped into three -Diagnostic HIV positive (A), HIV positive subjects (B) on antiretroviral drugs (HAART) and HIV negative subjects (C) used as control group. Quantitative estimation of HIV load was carried out with Real-Time PCR, while CD4 and CD8 T-lymphocyte estimation was carried out using C6 Acurri flow cytometer system. Statistical analysis was done with Mann-Whitney test, Kruskal-Wallis statistic and Dunn's Multiple Comparison Test at $p < 0.05$ as significant. There was significant reduction in mean CD4 T-lymphocyte counts in groups A (246.60±186.10) and B (255.40±168.70), while significant reduction in CD8 T-lymphocyte count occurred only in group A (449.20±273.50). CD4/CD8 ratio reduced significantly in both A (0.77±0.66) and B (0.65±0.47), while the mean viral load of group B (145591.00±259499.00) was significantly reduced when compared with that (24407.00±106479.00) of group A. Though there was down regulation of viral burden during highly active antiretroviral therapy (HAART), there was no corresponding increase in T-cell proliferation. This indicates that there might be a qualitative defect imparted on the progenitor cells of CD4 T-lymphocytes during progressive HIV disease.

**Key Words:** CD4 T-lymphocyte, antiretroviral therapy, HIV-infection, depletion and proliferation.

## INTRODUCTION

Human Immunodeficiency Virus (HIV) is the causative agent of the fatal condition known as Acquired Immunodeficiency Syndrome (AIDS) (Weiss, 1993; Douck *et al.,* 2009); a condition in man in which the immune system begins to fail, leading to life-threatening opportunistic infections. HIV infection occurs by a transfer of blood, semen, vaginal fluid, pre-ejaculate, breast milk, or other body fluids, contaminated with the virus, to a new host. The four major routes of transmission are unprotected sex, contaminated needles, breast milk and prenatal transmission. Screening of blood products for HIV has largely eliminated transmission through blood transfusion in the developed world, though in Nigeria, it still accounts for the second largest source of HIV infection after heterosexual sex (FRN, 2012).

However, it was estimated that from the discovery of HIV infection in 1981, to the year 2006, AIDS had killed more than 25 million people worldwide and infected about 0.6% of the world population. In 2005 alone, AIDS claimed an estimated 2.4 – 3.3 million lives, of which more than 570,000 were children. One third of these deaths occurred in sub-Saharan Africa, retarding economic growth and increasing poverty (UNAIDS and WHO, 2010). HIV infects primarily vital cells in the human immune system such as helper T-cells (especially $CD4^+$ T-cells), macrophages, and dendritic cells (Cunningham *et al.*, 2010). HIV infection leads to low level of $CD4^+$ T-cells through three main mechanisms which include direct viral killing of infected cells, increased rate of apoptosis in infected cells and killing of infected $CD4^+$ T-cells by CD8 cytotoxic T-lymphocytes. When $CD4^+$ T-cell number decline below a critical level, cell–mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. Most untreated people infected with HIV–1 eventually develop AIDS (Migueles and Connors, 2010).

Moreover, the failure of the immune system to contain HIV is related to the functional impairment of HIV-specific $CD4^+$ and $CD8^+$ T-cells that accompany the progression of HIV infection (Day *et al.*, 2006). It is the role of $CD4^+$ T-cells to signal other cells of the immune system to perform their respective functions (Deck *et al*., 2004; Ondoa *et al*., 2005) by secreting cytokines which are involved in signal transduction. The deterioration of T-cell responses or T-cell exhaustion in HIV infection involves the early loss of proliferative capacity, cytotoxic potential and the ability to produce interleukin-2 (Kostense *et al*., 2002).

Considering the importance of CD4 T-lymphocyte in the overall immune response, and devastative effect of HIV on CD4 T-lymphocytes during HIV disease, this study was designed to assess the depletive effect of HIV on CD4 T-lymphocytes and the capacity of highly active antiretroviral therapy (HAART) to restore the effect during drug intervention.

## MATERIALS AND METHODS

**Study Design:** A total of ninety (90) subjects were included in this study which has three groups with thirty (30) subjects in each study group. The groups include thirty (30) HIV-1 positive subjects that have not started receiving antiretroviral drugs, thirty (30) HIV-1 positive subjects on Highly Active Antiretroviral Therapy (HAART) and thirty (30) apparently healthy individuals that have tested negative for antibodies to HIV-1 and 2, used as control group. The test subjects were recruited from HIV/AIDS patients attending clinics at the University of Nigeria Teaching Hospital Ituku–Ozalla, Enugu State University Teaching Hospital (Park Lane), Annunciation Specialist Hospital Emene and Mother of Christ Specialist Hospital, all in Enugu, Nigeria. Control subjects were recruited from member of the public, students and staff of the hospitals stated above. Sample collection was completed within three months.

**Inclusion and Exclusion Criteria:** Qualification for recruitment into this study was based on the outcome of oral-interview/questionnaire, clinical and laboratory assessments. Each person recruited after the assessment, was included as a participant after an informed consent has been duly signed. The research considered only those on first line HAART drug combination and basically those on Zudovudine, lamivudine and nevirapine drug combination. With the approval of the ethics committee, the research was carried out at the Immunology Research Unit of the Department of Haematology and Immunology, University of Nigeria Teaching Hospital, Ituku–Ozalla, Enugu State.

**Ethical Consideration:** Ethical approval was obtained from the Medical Research Ethics Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. After critical study of the research proposal, a written approval was signed by the Committee. Each member of the recruited subjects read and signed the informed consent before enrollment as a participant.

**Blood Sample Collection:** A total of 5mls of blood was collected from each subject using dipotassium ethylendiamine tetra-acetic acid (EDTA) bottle. The samples were maintained at 2 to $8^0$C temperature range, and transferred immediately to the laboratory for analysis. After pre-analytical procedure, the sample aliquots were stored according to the analytical procedure for each variable. 500µl of the whole blood was delivered into 2ml cryovial and stored in liquid nitrogen for cluster of differentiation (CD) marker studies. The remaining whole blood was separated into plasma and packed cells. The plasma sample was frozen at -$85^0$C for viral load estimation.

**Sample Analysis:** HIV screening test was carried out with Genscreen ULTRA HIV Screening Kit for the detection of HIV P24 Antigen and Antibodies to HIV–1 and HIV–2 in Human Plasma by Enzyme Immunoassay (BIO–RAD). The



Genscreen™ ULTRA HIV Ag–Ab is an enzyme immunoassay based on the principle of the sandwich technique for the detection of HIV antigen and various antibodies associated with HIV–1 and / or HIV–2 virus in human serum or plasma. HIV–1/2 Confirmatory Test was done with Quali Code HIV–1/2 Kit, from Immunetics Inc. The Quali Code HIV–1/2 kit is a Qualitative Immunoblot Assay on the Western Blotting Principle. HIV RNA Extraction was carried out using Thermo Scientific Viral RNA Purification Kit from Thermo Fisher Scientific Inc while estimation of HIV Viral Load was done with Real Time-PCR method and TaqMan Chemistry. CD4 and CD8 T-lymphocyte levels were estimated using Accuri cytometer (C6 flow cytometer system). A beam of light (usually laser light) of a single wavelength is directed onto a hydrodynamically focused stream of liquid to form both forward and side scatter. The combination of scattered and fluorescent light is picked up by a detector system to analyze and differentiate various types of structures of individual particle.

**Statistical Analysis:** Kruskal-Wallis statistic and Dunn's Multiple Comparison Test were used to test for levels of significance in CD4 and CD8 T-lymphocyte concentrations, while Mann-Whitney test was used to test for viral load. The levels of significance were set at $p < 0.05$.

## RESULT

The results showed that the mean value of $CD4^+$ T-lymphocyte count in diagnostic HIV positive subjects (246.60±186.10) was significantly reduced ($P<0.05$) in comparison with that of the HIV seronegative control subjects (996.40 ± 207.10) (*see* Table 1).

**Table 1: $CD4^+$ T-lymphocyte levels in diagnostic HIV positive subjects, HIV positive subjects on ARV drugs and HIV Seronegative control subjects.**

| Parameters (cells/µl) | Diagnostic HIV | ARV Drugs | HIV Seronegative |
|---|---|---|---|
| Median | 177.00 | 202.50 | 976.50 |
| Mean | 246.60 | 255.40 | 996.40 |
| Standard Deviation | 186.10 | 168.70 | 207.10 |
| Minimum | 85.00 | 97.00 | 681.00 |
| Maximum | 920.00 | 819.00 | 1366.00 |
| Normality | Yes | Yes | Yes |

Table's statistics for Dunn's Multiple Comparison Test: (1) Diagnostic (246.60±186.10) vs ARV (255.40±168.70); Difference in rank sum =-3.367 ($P > 0.05$). (2) Diagnostic (246.60±186.10) vs Control (996.40±207.10); Difference in rank sum = -45.58 ($P < 0.05$). (3) ARV (255.40±168.70) vs Control (996.40±207.10); Difference in rank sum = -42.22 ($P < 0.05$).

Also, the mean $CD4^+$ T-lymphocyte count of HIV positive subjects on antiretroviral drugs (255.40±168.70) showed statistically significant difference ($P<0.05$) from that of the control group (996.40±207.10). However, at 5% level of significance, there was no statistically significant difference observed when the mean $CD4^+$ T-lymphocyte count of diagnostic HIV positive subjects (246.60±186.10) was compared with that of the HIV positive subjects on antiretroviral drugs (255.40±168.70) ($p>0.05$) (*see* Table 1).

On the other hand, the mean $CD8^+$ T-lymphocyte count of diagnostic HIV positive subjects (449.20±273.50) in comparison with that of the HIV positive subjects on ARV drugs (463.80±208.10) showed no significant difference ($p>0.05$) (*see* Table 2).

Furthermore, there was no statistically significant difference ($P>0.05$) in the mean $CD8^+$ T-lymphocyte level in HIV positive subjects on ARV drugs (463.80±208.10) and that of the control group. On the contrary, there was a significant difference ($p<0.05$) between the mean $CD8^+$ T-lymphocyte counts of diagnostic HIV positive subjects (449.20±273.50) and that of HIV seronegative controls (613.20 ± 258.20) (*see* Table 2).

The mean CD4/CD8 T-lymphocyte ratios of diagnostic HIV subjects (0.767±0.661) and HIV seronegative control subjects (1.867±0.723) as presented in table 3 below, showed a statistically significant difference ($P<0.05$) at 5% level of

significance. Also, between HIV positive subjects on ARV drugs and HIV seronegative control groups, the mean CD4/CD8 ratios (0.647±0.471 and 1.867±0.723 respectively) was significantly different (P<0.05) at 5% level of significance. On the other hand, the comparison between the mean CD4/CD8 T-lymphocyte ratios of diagnostic HIV positive subjects (0.767±0.660) and that of HIV positive subjects (0.647±0.471) on ARV drugs showed no significant difference (p>0.05).

**Table 2: $CD8^+$ T-lymphocyte levels in diagnostic HIV positive subjects, HIV positive subjects on ARV drugs and HIV Seronegative control subjects.**

| Parameters (cells/µl) | Diagnostic HIV | ARV Drugs | HIV Seronegative |
|---|---|---|---|
| Median | 405.50 | 407.50 | 562.00 |
| Mean | 449.20 | 463.80 | 613.20 |
| Standard Deviation | 273.50 | 208.10 | 258.20 |
| Minimum | 89.00 | 196.00 | 289.00 |
| Maximum | 1355.00 | 1048.00 | 1086.00 |
| Normality | No | Yes | No |

Table's statistics for Dunn's Multiple Comparison Test: (1) Diagnostic (449.20±273.50) vs ARV (463.80±208.10); Difference in rank sum = -3.883 (P > 0.05). (2) Diagnostic (449.20±273.50) vs Control (613.20±258.20); Difference in rank sum = -17.67 (P < 0.05). (3) ARV(463.80±208.10) vs Control (613.20±258.20); Difference in rank sum = -13.78 (P > 0.05)

**Table 3: CD4/CD8 T-Lymphocyte Ratio in Diagnostic HIV positive subjects, HIV positive subjects on ARV Drugs and HIV Seronegative control subjects.**

| Parameters | Diagnostic HIV | ARV Drugs | HIV Seronegative |
|---|---|---|---|
| Median | 0.50 | 0.50 | 1.65 |
| Mean | 0.77 | 0.65 | 1.87 |
| Standard Deviation | 0.66 | 0.47 | 0.72 |
| Minimum | 0.10 | 0.20 | 0.90 |
| Maximum | 2.70 | 1.90 | 3.40 |
| Normality Test | No | No | Yes |

Table's statistics for Dunn's Multiple Comparison Test: (1) Diagnostic (0.77±0.66) vs ARV (0.65±0.47); Difference in rank sum = 2.650 (P>0.05). (2) Diagnostic (0.77±0.66) vs Control (1.87±0.72); Difference in rank sum = -35.05 (P<0.05). (3) ARV(0.65±0.47) vs Control(1.87±0.72); Difference in rank sum = -37.70 (P<0.05)

**Table 4: Quantitative HIV virus in Diagnostic HIV Positive Subjects and HIV positive subjects on Antiretroviral Drugs (HAART).**

| Parameters (copies/ml) | Diagnostic HIV | ARV Drugs |
|---|---|---|
| Median | 20646.00 | 0.00 |
| Mean | 145591.00 | 24407.00 |
| Standard Deviation | 259499.00 | 106479.00 |
| Minimum | 1905.00 | 0.00 |
| Maximum | 926246.00 | 581442.00 |
| Normality | No | No |

* Medians were significantly different (P < 0.05)

The mean viral load of HIV positive subjects on ARV drugs (145591.00±259499.00) was significantly reduced when statistically compared with that (24407.00±106479.00) of diagnostic HIV positive subjects (p<0.05).



## DISCUSSION

Specific cytotoxic $CD8^+$ T-cells kill HIV-infected cells with the help of $CD4^+$ T-lymphocytes that are needed to prime $CD8^+$ T-cell responses and maintain both immunologic memory and cytolytic response. During acute HIV infection, $CD8^+$ T-cell count increases up to 20-folds with a vigorous specific anti-HIV response (Koup *et al*., 1994). The observed significant decrease of $CD8^+$ T-cell count in diagnostic HIV-positive subjects with respect to that of HIV-seronegative control group may be associated with the decrease in the $CD4^+$ T-lymphocyte counts of the diagnostic HIV-positive subjects. Depletion of $CD4^+$ T-cell count and its dysfunctional activity leads to decrease in the ability to recognize and respond to the HIV peptides on the surface of antigen presenting cells (APC), which may lead to low $CD4^+$ T-cell activation and adversely affect $CD8^+$ T-cell activation and proliferation, and specific cytotoxic $CD8^+$ T-cell killing of HIV-infected cells. This dysfunctional activity may give HIV allowance to proliferate, increase the viral load, infect more cells, deplete more $CD4^+$ T-cells and possibly cause permanent injury to the immune system.

Also, administration of antiretroviral drug (HAART) may cause reduction in the viral load, elevation of $CD4^+$ T-cells and possibly activate $CD8^+$ T-cells for proliferation and cytolytic activities (Kostense *et al.*, 2002). This may be the reason for the observed non-significant difference between the $CD8^+$ T-cell count in HIV-positive subjects on antiretroviral drugs and that of the HIV-seronegative control group in this study. Such finding may imply that highly active antiretroviral therapy has tried to restore the immune response by up-regulating the $CD4^+$ T-cell and cytotoxic T-lymphocytes quantitatively and qualitatively. This may probably be emphasized by the observed significant reduction in the plasma viral load of the HIV-positive subjects on antiretroviral drugs when compared with those of the diagnostic HIV-positive subjects. Though there was an elevation of mean CD4 T-lymphocyte count during antiretroviral drug intervention, there was no statistical significant deference when compared with that of the Diagnostic HIV positive subjects.

Moreover, the number of circulating $CD4^+$ T-cells is widely used to monitor the degree of immune suppression in HIV infection and provides a predictor of the immediate risk for opportunistic illness (Chiappini *et al*., 2006). In early HIV infection, $CD8^+$ T-cell number tends to increase, reflecting expansion of memory $CD8^+$ T-cells, particularly HIV-specific $CD8^+$ T-cells. In contrast to this, the proportion of naive $CD8^+$ T-cells tend to fall in early infection but absolute number of $CD8^+$ T-cell do not fall until HIV disease progresses (Chinen *et al*., 2001). In the present study, the CD4/CD8 ratio significantly reduced in both diagnostic HIV-positive subjects and HIV positive subjects on antiretroviral therapy compared with the CD4/CD8 ratio of HIV-seronegative control subjects. This finding may be as a result of persistent destruction of $CD4^+$ T-cells throughout the stages of HIV disease (Day *et al*., 2006). Absolute fall in $CD4^+$ T-cells count in relation to $CD8^+$ T-cells count may result to the fall in CD4/CD8 ratio observed in this study. This may be attributed to the infection of HIV virus to the progenitor cells of CD4 T-lymphocytes especially during progressive HIV infection. Such infection, with consistent activation and cytolysis may have impacted a permanent injury to proliferative capacity of the progenitor T-helper cells. Clearance of the virus from the system may not be good enough to effect a complete restoration of immune response. This observation may contribute to the fact that a stable HIV patient who is on antiretroviral drug goes back to a critical condition almost immediately after withdrawal of drug intervention which was observed from defaulters or non-adherents.

## CONCUSION

HIV infection causes depletion of CD4 T-lymphocyte. Though, clearance of HIV particles or reduction in viral load by antiretroviral drug intervention lead to increase in the mean CD4 T-lymphocyte count and restoration of physiological condition of the subjects, there was no significant difference between the mean CD4 T-lymphocyte count of HIV positive subjects on antiretroviral drug and that of the Diagnostic HIV positive subjects. There may be a proliferative defect inflicted on the progenitor cells by the virus infection during progressive HIV disease.

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**AUTHORS' CONTRIBUTIONS**

All the authors played significant roles in the different stages that culminated to this research report. These include field work/sample collection, literature search, laboratory analysis, data analysis and article drafting/revision.