THE RELATIONSHIP BETWEEN IL-2 CYTOKINE SECRETION AND CD4 T-LYMPHOCYTE DEPLETION IN PEOPLE LIVING WITH HIV/AIDS, IN ENUGU, NIGERIA.

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ABSTRACT

This study evaluated the relationship between IL-2 cytokine secretion and CD4 T-lymphocyte depletion in People Living with HIV/AIDS (PLWHAS) in Enugu, Nigeria. Thirty (30) subjects were recruited into each of the three study groups:-Diagnostic HIV positive (A), HIV positive subjects on antiretroviral drugs (HAART) (B) and HIV negative control group (C). HIV load was estimated using Real-Time PCR with TaqMan Chemistry. Concentration of IL-2 cytokine was determined using ELISA technique, while CD4 T-lymphocyte count was done with C6 Acurri flow cytometer system. Levels of significance were tested using Mann-Whitney test, Kruskal-Wallis statistic and Dunn’s Multiple Comparison Test at p<0.05. IL-2 cytokine secretion increased significantly in group B (99.32±43.83) as compared to A (52.57±23.53) and C (66.50±29.90). A significant reduction in mean CD4 T-lymphocyte count in groups A (246.60±186.10) and B (255.40±168.70) was recorded. Similarly, the mean viral load of group B (145591.00±259499.00) was significantly reduced. Although there was elevation of IL-2 cytokine secretion during highly active antiretroviral therapy (HAART), with down regulation of viral burden, no corresponding increase in T-cell proliferation was recorded; indicating a possible qualitative defect in the IL-2 cytokines produced during antiretroviral drug intervention or poor expression of IL-2 receptors on CD4 T-lymphocytes.

Key Words: CD4 T-lymphocyte, IL-2 cytokine, Antiretroviral drug, HIV-infection and Viral load.

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INTRODUCTION

Interleukin-2 (IL-2) is a T-cell-derived cytokine known to be important in the regulation of growth and differentiation of T-cells, B-cells, natural killer cells, glioma cells and cells of the monocite lineage after specifically interacting with its receptors. High-affinity interleukin-2 receptor (IL-2R) is a heterotrimeric complex composed of the α-chain (CD25), β-chain (CD122), and the common γ-chain (CD132) (Refachi et al., 1998). Engagement of the IL-2R complex on activated T-cell initiates a complex signaling that can induce proliferation, increase survival as well as prime for activation-induced cell death (Refachi et al., 1998). 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 also 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.

Meanwhile, CD4 cells communicate with other cellular components of the immune system by either cell contact or the elaboration of soluble factors known as cytokines. There is a number of different cytokines, each having different and distinct actions. A bipolar Th1/Th2 concept was originally described in the mouse model, in which there was a division of CD4 cells into Th1 and Th2 cells based on the cytokine production from the respective cells (Gray et al., 2005). Th1 cells secrete cytokines that derive cell-mediated immunity. These cytokines are IL-1, IL-2, IL-6, IL-12, IL-15, tumor necrosis factor alpha (TNF-α), and interferon-γ. Th2 response derives a humoral immune response and stimulates B-cells. Th2 cytokines include IL-4, IL-5, and IL-10.
On the other hand, the failure of the immune system to contain HIV is related to the functional impairment of HIV–specific CD4\(^+\) and CD8\(^+\) T-cells that accompanies progressive HIV infection, a phenomenon which is referred to as T-cell exhaustion (Day et al., 2006). It is known that the CD4\(^+\) T-cells (T-helper cells) play a major role in the overall immune responses by signaling other cells in the immune system to perform their respective functions (Deck et al., 2004; Ondoa et al., 2005). The mechanisms leading to T-cell exhaustion in HIV infection are clearly complex and cannot be attributed to a single pathway. In HIV–1 infection, the deterioration of T cell responses involves the early loss of proliferative capacity, cytotoxic potential and the ability to produce interleukin–2 (IL-2), followed by a progressive loss of the ability to produce interferon gamma (INF-y) (Kostense et al., 2002).

Since nobody in our environment has looked at the effect of IL-2 cytokine level on CD4 T-lymphocyte proliferation and depletion during HIV infection, we designed this study to determine the relationship between CD4 T-lymphocyte depletion, IL-2 cytokine secretion and HIV viral load among people living with HIV and AIDS in Enugu, Nigeria.

**MATERIALS AND METHODS**

**Subjects / Study Design:** A total of ninety (90) subjects were recruited for this study. The subjects were divided into three groups with thirty (30) subjects in each study group. The groups included thirty (30) HIV-1 positive subjects that have not started receiving antiretroviral drugs and referred to as Diagnostic HIV positive subjects (A); thirty (30) HIV-1 positive subjects on Highly Active Antiretroviral Therapy (HAART) (B); and thirty (30) apparently healthy individuals that have tested negative for antibodies to HIV-1 and 2, and used as control group (C). 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 members of the public, students and staff of the hospitals stated above. Sample collection was completed within three months.

**Inclusion / Exclusion criteria:** This study included subjects 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 was 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 Approval:** Ethical clearance 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 into dipotassium ethylenediamine 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. Estimation of CD4 T-lymphocyte was carried out immediately while plasma sample was separated and kept in two aliquots for the estimation of IL-2 cytokine and viral load. The plasma samples were frozen at -85\(^0\)C.

**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. Estimation of IL-2 cytokine concentration was done with Abcam Human IL-2 ELISA Kit from Abcam UK. This is a solid phase sandwich enzyme linked-immunosorbent assay kit. CD4 T-lymphocyte level
was 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:** Levels of significance for IL-2 cytokine and CD4 T-lymphocyte concentrations were tested with Kruskal-Wallis statistic and Dunn’s Multiple Comparison Test while Mann-Whitney test was used to test for viral load. The levels of significance were set at $p < 0.05$.

**RESULTS**

The mean value of IL-2 cytokine secreted in the plasma of diagnostic HIV positive subjects (52.57 ± 23.53) differs significantly from that of HIV positive subjects on antiretroviral (ARV) drugs (99.32 ± 43.83) when compared statistically ($p<0.05$). There is also statistically significant difference between the mean values of IL-2 plasma levels of HIV positive subjects on ARV drugs (99.32 ± 43.83) and HIV seronegative control subjects (66.50 ± 29.90) ($p<0.05$). There was no significant difference observed when the mean values of IL-2 plasma level of diagnostic HIV positive subjects (52.57 ± 23.53) was statistically compared with that of the control subjects (66.50 ± 29.90).

In Table 2 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). Also, the mean CD4$^+$ T-lymphocyte count of HIV positive subjects on antiretroviral drugs (255.40±168.70) showed statistically significant, ($P<0.05$) when compared with that of the control group (996.40±207.10). 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), ($p>0.05$).

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

**Table 1:** Plasma IL–2 levels in diagnostic HIV positive subjects, HIV positive subjects on ARV drugs (HAART) and HIV Seronegative control subjects.

<table>
<thead>
<tr>
<th>Parameters (pg/ml)</th>
<th>Diagnostic HIV</th>
<th>ARV Drugs</th>
<th>HIV Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>47.26</td>
<td>107.7</td>
<td>65.82</td>
</tr>
<tr>
<td>Mean</td>
<td>52.57</td>
<td>99.32</td>
<td>66.50</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>23.53</td>
<td>43.83</td>
<td>29.90</td>
</tr>
<tr>
<td>Minimum (pg/ml)</td>
<td>34.26</td>
<td>17.06</td>
<td>17.79</td>
</tr>
<tr>
<td>Maximum (pg/ml)</td>
<td>143.10</td>
<td>180.80</td>
<td>130.20</td>
</tr>
<tr>
<td>Normality</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

For Dunn's Multiple Comparison Test - Diagnostic (52.57±23.53) vs ARV (99.32±43.83): Diff in rank sum = -30.98 ($P<0.05$); Diagnostic (52.57±23.53) vs Control (66.50±29.90): Diff in rank sum = -13.37 ($P>0.05$); ARV (99.32±43.83) vs Control (66.50±29.90): Diff in rank sum = 17.62 ($P<0.05$).
Table 2: CD4+ T-lymphocyte levels in diagnostic HIV positive subjects, HIV positive subjects on ARV drugs and HIV Seronegative control subjects.

<table>
<thead>
<tr>
<th>Parameters (cells/µl)</th>
<th>Diagnostic HIV</th>
<th>ARV Drugs</th>
<th>HIV Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>177.00</td>
<td>202.50</td>
<td>976.50</td>
</tr>
<tr>
<td>Mean</td>
<td>246.60</td>
<td>255.40</td>
<td>996.40</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>186.10</td>
<td>168.70</td>
<td>207.10</td>
</tr>
<tr>
<td>Minimum</td>
<td>85.00</td>
<td>97.00</td>
<td>681.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>920.00</td>
<td>819.00</td>
<td>1366.00</td>
</tr>
<tr>
<td>Normality</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

For Dunn's Multiple Comparison Test -Diagnostic (246.60±186.10) vs ARV (255.40±168.70): Diff. in rank sum = 3.367 (P>0.05); Diagnostic (246.60±186.10) vs Control (996.40±207.10): Diff. in rank sum = -45.58 (P<0.05); ARV (255.40±168.70) vs Control (996.40±207.10): Diff. in rank sum = -42.22 (P<0.05)

Table 3: Quantitative HIV virus in Diagnostic HIV Positive Subjects and HIV positive subjects on Antiretroviral Drugs (HAART).

<table>
<thead>
<tr>
<th>Parameters (copies/ml)</th>
<th>Diagnostic HIV</th>
<th>ARV Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>20646.00</td>
<td>0.00*</td>
</tr>
<tr>
<td>Mean</td>
<td>145591.00</td>
<td>24407.00</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>259499.00</td>
<td>106479.00</td>
</tr>
<tr>
<td>Minimum</td>
<td>1905.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>926246.00</td>
<td>581442.00</td>
</tr>
<tr>
<td>Normality</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Medians were significantly different

DISCUSSION

In this study, low level of plasma IL-2 cytokine observed in Diagnostic HIV-positive subjects in spite of high viral load and constant immune challenges by the viral particles may be attributed to the observed low CD4+ T-lymphocyte count in the same group. It is known that CD4+ T-cells secrete IL-2 cytokines which in turn activates the proliferation of both CD4+ and CD8+ T-lymphocytes (Refachi et al., 1998). Low level of CD4+ T-lymphocyte may possibly lead to low synthesis of IL-2 cytokine and low activation of both CD4+ and CD8+ T-lymphocytes. This may further reduce the capacity of the immune system to modulate the proliferative rate and expansion of HIV virus in the system. The significant increase in plasma IL-2 level of HIV-positive subjects on ARV drugs observed in this study in comparison with both diagnostic HIV-positive and HIV-seronegative control groups may then be attributed to the impact of highly active antiretroviral drugs (HAART) on the viral particles.

However, CD4 molecules found on the surface of helper T-lymphocytes, and other CD4+ - cells like monocytes, macrophages and dendritic cells help to stabilize the binding of T-lymphocytes to the major histocompatibility complex (MHC) class II on the antigen presenting cells (APC). Activation of T-helper cells through such binding orchestrates the body’s antigen-specific immune response by coordinating B-lymphocyte production of antibodies, secretion of cytokines by various immune cells and induction of cytotoxic lymphocyte response to the antigens. These functions make CD4+ T-lymphocytes critical elements of the immune system (Wilson et al., 2004; Vajpayer et al., 2005; Vajpayer et al., 2009). Low level of CD4 T-lymphocytes found in Diagnostic HIV positive subjects may account for the increase in viral load observed in this group.

In addition, HIV has direct and indirect cytolytic effect on both mature CD4+ T-cells and on the progenitor cells which is the reason for progressive exhaustion that accompanies the loss of capacity to produce new cells (Antran, 2000; Brenchley et al.,...
Contrary to this expectation, there was no significant increase in CD4 T-lymphocyte count in HIV positive subjects on antiretroviral drugs when compared with that of the Diagnostic HIV positive subjects even with a very significant increase in IL-2 cytokine secretion. It may imply that reduction or clearance of HIV virus in the blood may induce secretion of IL-2 cytokine even with little increase in CD4 T-lymphocyte count. The IL-2 cytokine produced under this condition may not have proliferative capacity to affect a corresponding increase in CD4 T-lymphocyte count. This observation may be a proliferative dysfunction induced on the CD4 T-lymphocyte progenitor cells by HIV particles during progressive HIV infection.

CONCLUSION

HIV infection causes decrease in IL-2 cytokine secretion. Clearance of HIV particles or reduction in viral load by antiretroviral intervention leads to increase in both CD4 T-lymphocyte count and IL-2 cytokine secretion. But increase in IL-2 cytokine secretion during antiretroviral therapy does not lead to a corresponding increase in CD4 T-lymphocyte count. This may indicate a qualitative impairment of the IL-2 cytokines produced during antiretroviral therapy or poor expression of IL-2 cytokine receptor (IL-2R) complex, on activated T-helper cells.

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REFERENCES


**AUTHORS’ CONTRIBUTIONS**

Ohotu, E. O., was responsible for the design of this work and supply of some literature material. Onyemelukwe, N was responsible for organization of the manuscript. All authors (Ohotu, E. O., Onyemelukwe, N., Odurukwe, O. U and Ezema, C. I.) contributed to the completion of this study and were actively involved in the presentation of this manuscript.