Background: Human Immunodeficiency Virus (HIV) is associated with a decline of immunity and progression to Acquired Immunodeficiency Syndrome (AIDS). This current study was aimed at evaluating the serum level of Interleukin-8 (IL-8), CD4+ and CD8+ T-cells counts among newly diagnosed Human Immunodeficiency Virus (HIV)-positive adults in Sokoto-Nigeria.

Materials and methods: A total of 60 adults were enrolled into the study, comprising of 30 newly diagnosed HIV seropositive subjects and 30 age-and sex-matched apparently healthy controls. CD4+ and CD8+ T-cells were enumerated using flow cytometry. Serum IL-8 was analyzed using ELISA kit. Data were analyzed using SPSS 20.0 statistical package. A p-value ≤ 0.05 was considered significant in all statistical comparisons.

Results: The mean of CD4+ (632.63 ± 34.83 cells/μl), CD8+ (579.98 ± 30.74 cells/μl) and CD4+/CD8+ ratio (1.10 ± 0.03 cells/μl) were significantly higher (p < 0.05) in controls compared to newly diagnosed HIV patients (160.60 ± 29.47 cells/μl, 521.10 ± 46.34 cells/μl and 0.32 ± 0.52 respectively. The mean of IL-8 (6.56 ± 0.09 pg/ml) was significantly lower (p < 0.05) in controls compared to newly diagnosed patient IL-8 (6.60 ± 0.07 pg/ml). There were insignificantly negative correlation between IL-8 and CD4+ (r = -0.064, p > 0.05) as well as CD8+ (r = -0.074, p > 0.05) in controls. And conversely, an insignificant positive correlation occurred between IL-8 and CD4+ (r = 0.025, p > 0.05) as well as CD8+ (r = 0.016, p > 0.05) in newly diagnosed HIV patients. Moreover, it has also shown a statistically insignificantly positive correlation between IL-8 and CD4+/CD8+ ratio (r = 0.039, p > 0.05) in controls compared to newly diagnosed HIV patients where it shows a statistically insignificant negative correlation between the IL-8 and CD4+/CD8+ ratio (r = -0.076, p > 0.05).

Conclusion: It could be concluded that, both CD4+, CD4+/CD8+ ratio in newly diagnosed HIV patients are significantly decrease compared to controls (p < 0.05), while serum IL-8 was significantly increased, the serum levels of IL-8 did not correlate with CD4+ T-cells and CD8+ T-cells population, as well as CD4+/CD8+ ratio.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a retrovirus belonging to the family Lenti viruses, which are responsible for chronic and long lasting infections [1]. HIV pandemic is a major public health problem with an estimated 33 millions people living with the virus globally [2]. Available data indicated that sub-Saharan Africa is bearing the greatest brunt of the global HIV scourge with an estimated 25.8 millions people equivalent to 70% out of the number of people living with HIV.
against T-cells and basophils. Neutrophil adhesion to and inflammation, in

major property of IL-8 during the inflammation. SB225002 also markedly reduces HIV-1 replication. A actions of IL-8 with a small molecule inhibitor of CXCR2 HIV-1 replication in macrophages and T-cells. Blocking the block binding to the receptors CXCR1 and CXCR2, can inhibit activities are mediated through MHCI and is responsible for coordinating Cytotoxic Immune Responses (CD8+), while the TH2 subset acts through exogenous pathways, where it tries to prevent the body from being infected by virus, while increasing secretion of antibody. Individuals, who have a dominant, TH1 but infected with HIV tend to survive longer than those recessive TH1. It’s on record that CD8+ T-cells has the capacity to inhibit HIV infection through, HLA-restricted cytolysis as well as suppressive activity mediated by the secretions of multiple suppressive factors collectively referred to as CD8+. Antiviral Factor (CAF) [5]. Interleukin-8 (IL-8, CXCL8) is a member of the proinflammatory CXC cytokine family implicated in mediation of inflammatory responses, including angiogenesis, leukocyte degranulation and cell migration [6]. CXC chemokines are small proteins (8-12 kDa) that contain a conserved CXC residue motif (C-cysteine, X-any other residue) proximal to the N-terminal region of the protein [7]. Biologic actions of IL-8 include the induction of respiratory burst [8], induction of chemotaxis of neutrophils. T- cells [9] and basophils [10]. Promoting release of lysosomal enzymes from neutrophils, and enhanced killing of various microorganisms including M. tuberculosis [11].

Lane, et al. [12] Shows that increased levels of IL-8 are present in the lymphoid tissue of patients with AIDS. Antibodies that neutralize IL-8 activity, and antibodies that block binding to the receptors CXCR1 and CXCR2, can inhibit HIV-1 replication in macrophages and T-cells. Blocking the actions of IL-8 with a small molecule inhibitor of CXCR2 SB225002 also markedly reduces HIV-1 replication. A major property of IL-8 during the inflammatory process is chemotaxis of targe T-cells to the site of inflammation, in particular neutrophils. IL-8 also has chemotactic activity against T-cells and basophils. Neutrophil adhesion to and transmigration across the endothelium are regulated by IL-8 and once neutrophils arrive to the site of inflammation, IL-8 further stimulates those cells to carry out phagocytosis, thus increasing the efficiency of tissue repair. Studies have also shown that IL-8 also has other immunomodulatory effects including the ability to induce matrix metalloproteinase-9 expression, release of TNF-Related Apoptosis-Inducing Ligand (TRAIL) and prime respiratory burst in neutrophils [13].

This research study was designed to evaluate serum levels of IL-8, plasma CD4+ T-cell and CD8+ T-cell counts in newly diagnosed HIV patients and compared with that in the healthy controls. This study may help to find out if the levels of IL-8 is in any way related to the severity of HIV infection, which may predict the HIV disease progression to AIDS, thus exploring the possibility for effective management of these patients.

**MATERIALS AND METHODS**

**Study location**

This research study was done in the State Specialist hospital Sokoto, and School of Medical Laboratory Sciences, Immunology Department, Usmanu Danfodiyo University Sokoto-Nigeria. The State has a semi-arid climatic condition, with a vegetation, which is largely of Sudan Savannah. The annual rainfall is in the range of 550 - 1400 mm, with a temperature ranges 140C to 500C in an extreme condition SSBD, 2007).

**Study subjects**

A total of Thirty (30) newly diagnosed adults male and female HIV patients, attending ART Clinic in some major hospitals in the state and Thirty (30) age-and sex-matched apparently healthy controls who passed all the inclusion criteria were randomly recruited into the study informed consent was obtained from each prior to the commencement of the study.

**Ethical approval**

The ethical approval for this research was sought from the Ethics and Research Committee of Specialist hospitals Sokoto. The research was carried out in accordance with the declaration of Helsinki concerning the ethical principles for medical research involving human subjects.

**Study design**

A cross sectional study where eligible HIV-positive male and female patients attending some selected hospital Sokoto, were included in the study. All the HIV patients were evaluated clinically by the Consultant Physicians and relevant clinical data obtained. Eligible subjects were assigned to into two groups, Group A consisting of 30 HIV-negative controls and Group B consisting of 30 newly diagnosed HIV patients.
Anthropometric measurements

**Body weight:** At enrolment, the subjects was weighted with minimum clothing to the nearest 0.1kg by using a regularly calibrated weighing health scale; model ZT 120 (manufactured by Seca Gmbh and Co., Germany).

**Height:** The heights of the subjects were measured by using a calibrated Stadiometer, model 220 (manufactured by Seca Gmbh and Co., Germany). For height measurement, the subject stand erected and barefooted on a Stadiometer with a movable head piece. The head piece was levelled with skull vault and height recorded to the nearest 0.5 cm.

**Body mass index:** Body Mass Index (BMI) for each subject was calculated using the following formula:

\[
BMI (kg/m^2) = \frac{Body\ Weight\ (kg)}{Height\ (m)^2}\.
\]

Blood samples collection and processing

From each selected subject, a total of five millilitres (5.0ml) of venous blood specimen was collected using a sterile vacutainer blood specimen bottles, holder and needle. Three millilitres (3.0ml) of blood specimen was collected into a sterile plain vacutainer blood specimen bottle, and allowed to clot at room temperature and later centrifuged at 3000rpm/min for 5 minutes, then obtained a clear unhaemolyzed serum. The serum was harvested into sterile plain vacutainer blood specimen bottle, and allowed to clot at room temperature and later centrifuged at 3000rpm/min for 5 minutes, then obtained a clear unhaemolyzed serum. The serum was harvested into sterile vacutainer blood specimen bottles, holder and needle. Two millilitres (2.0ml) of the blood specimen was collected into a sterile EDTA vacutainer blood specimen bottle, and used to re-determined and confirmed HIV–status and for the enumeration of CD4⁺ T-cell and CD8⁺ T-cell count within 3 hours of the blood sample collection.

Laboratory analysis

**Evaluation of CD4⁺ and CD8⁺ cell count:** The CD4⁺ or CD8⁺ T-Cells were enumerated using Cyflow Counter manufactured by Partec, Munster, Germany, which operate on the principle of flow cytometry method by Cassens.

**Determination of CD4⁺/CD8⁺ ratio:** The CD4⁺/CD8⁺ Ratio was calculated using, the number of CD4⁺ T-cells count (cells/µl of blood) / Number of CD8⁺ - cells count (cells/µl of blood).

**Evaluation of serum Interleukin-8 (IL-8):** Serum level of Interleukin-8 (IL-8) was measured using quantitative ELISA method and the kit was procured from Invitrogen Corporation KHC0081. The analysis was done in strict adherence to the manufacturer’s instructions. The result was read spectrophotometrically using Elisa reader at a wavelength of 450nm.

Statistical analysis

The data obtained was analyzed using Microsoft Office Excel 2013 and Statistical Package for Social Sciences (SPSS) version 20. The results were expressed as mean ± SEM. Group comparisons was made using one-way Analysis of Variance (ANOVA), correlation analysis was done between the groups, paired comparisons was carried out using the Student’s T-test, and p-value of equal to or less than 0.05 (\(p \leq 0.05\)) was considered as significant.

Presentation of results

**Socio-economic and demographic characteristic of the study subjects:** Table 1 shows the gender, marital status, occupation and educational level in the study groups, among which 20(66.7%) and 15(50.0%) were males for controls and newly diagnosed HIV patients respectively. While females were 10(33.3%) and 15(50.0%) for controls and newly diagnosed HIV patient respectively. The result also indicated that 6(13.3%) of the subjects in controls were married as compared with 16(53.3%) in newly diagnosed HIV patients. Most of the subjects in controls group were students 17(56.7%) as compared to newly diagnosed, where the highest percentage of them were marketers 13(43.3%). Moreover, it has also shown that in control group the highest educational level attained (tertiary) belong to the control group with 25(83.3%) as compared to newly diagnosed HIV patient group where majority were illiterate 16(53.3%).

**Serum IL-8, CD4⁺, CD8⁺ and CD4⁺/CD8⁺ ratio of the study subjects:** The mean of CD4⁺ (632.63 ± 34.83 cells/µl), CD8⁺ (579.98 ± 30.74 cells/µl) and CD4⁺/CD8⁺ ratio (1.10 ± 0.03 cells/µl) were significantly higher (\(p < 0.05\)) in controls compared to newly diagnosed patients (160.60 ± 29.47 cells/µl, 521.10 ± 46.34 cells/µl and 0.32 ± 0.52 respectively), as shown in table 2. The mean of IL-8 (6.56 ± 0.09 pg/ml) was significantly (\(p < 0.05\)) lower in controls as compared to newly diagnosed patient IL8 (6.60 ± 0.07 pg/ml).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n = 30)</th>
<th>Newly diagnosed HIV patients (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20(66.7%)</td>
<td>15(50.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>10(33.3%)</td>
<td>15(50.0%)</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>26(86.7%)</td>
<td>9(30.0%)</td>
</tr>
<tr>
<td>Married</td>
<td>4(13.3%)</td>
<td>16(53.3%)</td>
</tr>
<tr>
<td>Widowed</td>
<td>0(0.00%)</td>
<td>5(16.7%)</td>
</tr>
<tr>
<td>Divorced</td>
<td>0(0.00%)</td>
<td>0(0.00%)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>8(26.6%)</td>
<td>13(43.3%)</td>
</tr>
<tr>
<td>Student</td>
<td>17(56.7%)</td>
<td>3(10.0%)</td>
</tr>
<tr>
<td>Civil servant</td>
<td>3(10.0%)</td>
<td>5(16.7%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>2(6.7%)</td>
<td>11(36.7%)</td>
</tr>
<tr>
<td>Educational Level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1(3.3%)</td>
<td>1(3.3%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>4(13.3%)</td>
<td>9(30.0%)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>25(83.3%)</td>
<td>4(13.3%)</td>
</tr>
<tr>
<td>No formal education</td>
<td>0(0.00)</td>
<td>16(53.3%)</td>
</tr>
</tbody>
</table>
Table 2: Serum IL-8, CD4+, CD8+ counts and CD4+/CD8+ ratio of the study subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 30)</th>
<th>Newly diagnosed HIV patient (n = 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+(cells/μl)</td>
<td>632.63 ± 34.83</td>
<td>160.60 ± 29.47</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD8+(cells/μl)</td>
<td>579.98 ± 30.74</td>
<td>521.10 ± 46.35</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CD4+/CD8+(cells/μl)</td>
<td>1.10 ± 0.03</td>
<td>0.33 ± 0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-8(pg/ml)</td>
<td>6.56 ± 0.09</td>
<td>6.60 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM; CD4+ = Cluster of Differentiations Four; CD8+ = Cluster of Differentiations Type Eight; pg/ml = Picogram per Miles; μl = Microlitre.

Table 3: Correlation of serum IL-8 with CD4+ counts, CD8+ counts and CD4+/CD8+ ratio.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 30)</th>
<th>Newly diagnosed HIV patients (n = 30)</th>
<th>r - value</th>
<th>p - value</th>
<th>r - value</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+(cells/μl)</td>
<td>-0.064</td>
<td>&lt;0.05</td>
<td>0.025</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8+(cells/μl)</td>
<td>-0.074</td>
<td>&lt;0.05</td>
<td>0.016</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+/CD8+(cells/μl)</td>
<td>0.039</td>
<td>&lt;0.05</td>
<td>-0.076</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation studies of serum IL-8, CD4+4, CD8+ and CD4+/CD8+ ratio: There were insignificant negative correlation between IL-8 and CD4+ (r = -0.064, p > 0.05) as well as CD8+ (r = -0.074, p > 0.05) in controls. And conversely, an insignificant positive correlation occurred between IL-8 and CD4+ (r = 0.025, p > 0.05) as well as CD8+ (r = 0.016, p > 0.05) in newly diagnosed HIV patients, as shown in table 3. Moreover, it has also shown a statistically insignificant positive correlation between IL-8 and CD4+/CD8+ ratio (r = 0.039, p > 0.05) in controls compared to newly diagnosed HIV patients where it shows a statistically insignificant negative correlation between the IL-8 and CD4+/CD8+ ratio (r = -0.076, p > 0.05).

Correlation of anthropometric and immune parameters in controls and patients: There was no significant negative correlation of age with CD4+ and CD8+ counts, but there was insignificant positive correlation of age with CD4+/CD8+ ratio and IL-8. However, there was an insignificant correlation of BMI with CD4+, CD8+ and CD4+/CD8+ ratio.

**DISCUSSION**

Human Immunodeficiency Virus (HIV) and its subtypes are retroviruses and the etiologic agents of acquired Immunodeficiency Syndrome (AIDS). According to De Cock, et al. [14] and Maarten, et al. [15] HIV once entered into the body destroys the immune system and progresses to AIDS, hence all HIV infected persons are at risk of illnesses and death from opportunist infections and neoplastic complications due to inevitable manifestation of AIDS.

The level of IL-8 in this research was found to be highly upregulated compared to the controls. This elevation in the level of IL-8 could be attributed to increases in inflammation responses evident in newly diagnosed HIV patient. This research finding is consistent with the report of Robert, et al. [17] and Kolacinski, et al. [18]. Whose reported the there is a significant increase in the level of IL-8, in the HIV infected individuals. There was no significant correlation between the Anthropometric and Immune parameters in both controls and Patients evaluated in this research study.

Statistically insignificant correlation between high level of IL-8 and lower number of CD4+ and CD8+ T-cells in newly diagnosed HIV patients compared to controls, was also demonstrated in this study. This is consistent to the report of Pananghat, et al. [19], who also reported the same statistical in-significant correlation between the levels of IL-8, and that of CD4+ and CD8+ T-cells, but the findings is contrary to the report of Ping, who reported a statistical significance. The destruction of CD4+ T lymphocytes has generally been observed and acknowledged as a direct consequence of the immune-pathogenesis in HIV-1 infection, hence the depletion in their numbers as shown by this study.

During HIV infection IL-8 plays an important role in the recruitment of CD4+ positive T-cells to the lymph nodes, thus generating more targets for viral replication, this is partly responsible for the elevated level of IL-8 in the serum of early HIV infected patients as demonstrated by this study. Matsumoto, et al. [20] have reported elevated serum levels of IL-8 in HIV-1 infected patients. Levels of IL-8 have also been reported to be significantly elevated in Cerebrospinal Fluid (CSF) of HIV-1 infected patients with cryptococcal
meningitis and in the plasma of asymptomatic HIV-1-infected individuals, although not in AIDS patients.

IL-8 is an inflammatory and antimicrobial cytokine, it is a potent chemotactic factor for T-cells, NK cells, neutrophils and basophils [21]. IL-8 is upregulated and constitutively secreted following in vitro HIV-infection of MDM [22], but reduced in the chronically infected U937 monocytic cell line [23]. Elevated levels of IL-8 have been also observed in patients with HIV-1 infection, both in patients’ serum [24] and bronchoalveolar fluid [25] HIV-1 infection at all stages of the disease is associated with chronic immune activation and dysfunctional cytokine production.

Increased production of proinflammatory cytokines (IL-1, IL-6, IL-8 and TNF-α) are also thought to activate HIV-1 replication and maintain active HIV-1 expression via binding of NF-κB to LTR.

CONCLUSION/RECOMMENDATION

In conclusion, it should be point out that, serum interleukin 8 increased significantly in newly diagnosed HIV patients and this is associated with significant reduction in the CD4 T-cells, CD8 T-cells, and CD4+/CD8 T-cells ratio in newly diagnosed HIV patients.

More research should be undertaken, to actually establish the precise role of IL-8 in HIV disease progression and pathogenesis.

ACKNOWLEDGEMENT

We wish to acknowledge the efforts of Immunology Department, Academic and technical staff of School of Medical Laboratory Sciences, and that of state specialist Hospital sokoto, for all their individual and collective efforts in making this research a success.

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