T-LYMPHOCYTE SUB-SET (CD_4^+ AND CD_8^+) COUNTS AND SERUM INTERLEUKIN 8 (IL-8) IN HIV INFECTED ADULTS ON HAART IN SOKOTO, NIGERIA.

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ABSTRACT

Background: Infection by Human Immunodeficiency Virus disrupts the immune system through generalized immune activation and CD_4^+ T cell depletion.

Aim: The study was designed to evaluate the Serum level of Interleukin-8 (IL-8), Lymphocyte (CD_4^+ and CD_8^+) numbers in Human Immunodeficiency Virus (HIV) Patients on HAART in Specialist Hospital Sokoto.

Materials and Methods: The study included a total of 60 adult subjects (30 HIV seropositive subjects on HAART and 30 apparently healthy controls). CD_4^+ and CD_8^+ T cells were counted using flow cytometry. Serum IL-8 was analyzed using ELISA kit (KHC0081). Data were analyzed using SPSS version 20.0. A p-value $\leq$ 0.05 was considered statistically significant.

Result: The result indicated significant decrease of CD_4^+ and CD_4^+/ CD_8^+ ratio in HIV on HAART as compared to control ($p<0.05$). CD_8^+ count also decrease in HIV on HAART when compared to control but statistically not significant ($p>0.05$) while there was significant increase in serum IL-8 level in HIV on HAART as compared to control ($p<0.05$). Age and BMI had no effect on IL-8, CD_4^+, CD_8^+ and CD_4^+/ CD_8^+ ratio. The serum IL-8 is significantly increased in HIV patients on treatment with HAART. CD_4^+ and CD_8^+ T cell counts significantly decreased in HIV patients on treatment with HAART.

Conclusion and Recommendation: There is need of Periodic evaluation of serum interleukin-8 in patients with HIV, to enable professional counseling of patients at high risk of developing AIDS.

Keywords: CD_4^+, CD_8^+, HAART, HIV, IL-8, Sokoto.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a global pandemic which is becoming a serious health problem in Sub-Saharan Africa, Nigeria inclusive. HIV is a lent virus, a member of retrovirus family that causes acquired immunodeficiency syndrome (AIDS). HIV was first recognized in the summer of 1981 but has now assumed a pandemic proportion (Oluboyo *et al.*, 2006). In 1998, HIV was reported as the fourth leading cause of death worldwide with estimated 2.5 million deaths annually (Abdulsalami and Tekena, 2006).By the end of 2005, the estimated number of people living with HIV and AIDS has risen to 38.6 million with about 2.8 million deaths (UNAIDS/WHO, 2006). This has significantly reduced to 34 million people living with HIV/AIDS with about 1.8 million deaths in 2010 (UNAIDS, 2011). In 2011, 1.7 million people died from AIDS-related causes worldwide (UNAIDS, 2012). This represents a 24% decline in AIDS-related mortality compared with 2005 when 2.3 million deaths occurred. However, more than 25 million people are living with HIV/AIDS since 1981 (UNAIDS/WHO, 2006). In 2016, the estimated number of people living with has reached 39.78 million worldwide (CDC, 2016).

A report by the global HIV/AIDS pandemic (2006) showed that approximately 64% of world populations living with HIV are in the sub-Saharan Africa.

In Nigeria, a total of 3.1 million people are living with HIV as at the end of 2011 and about 300, 000 new infections are occurring annually (UNAIDS, 2011). HIV/AIDS prevalence rate in Nigeria dropped from 5.1% to 3.4% (NACA, 2015).

Human immunodeficiency virus (HIV) infects cells which bear its receptor, the CD_4^+lymphocytes molecule, as well as mononuclear phagocytes, peripheral blood monocytes, and tissue macrophages (Jean *et al*, 1989). IL-8 is produced by macrophages in response to HIV-1 infection (Lane *et al.,* 2001). Therefore, evaluating the serum levels of IL-8 together with $CD_4^+$and CD_8^+ estimation amongst HIV patients will provide a useful guide in the management of HIV infection.

Interleukin-8 (IL-8) was identified in 1987 as a novel type of neutrophil-activating cytokine (Baggiolini *et al*., 1992). IL-8 is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells.

The IL-8 cDNA encodes a 99- amino acid precursor protein with a signal sequence, which is cleaved to yield mainly 77- or 72-residue mature protein (Matsushima *et al.*, 1988). IL-8 is further processed at the NH_2 terminus yielding different truncation analogs (77-, 72-, 71-, 70-, 69-amino acid forms). The truncation is caused by proteases that are released fromCXCL8 (CXC chemokine ligand-8)-secreting cells or by accessory cells (Padrines *et al*., 1994) and the occurrence of the NH_2-terminal forms depends on the producer cells and culture conditions (Van Damme *et al*., 1990).

It was demonstrated that elevated levels of IL-8 are present in the sera and lungs of HIV-1 infected patients (Lane *et al.*, 2001). They also showed that exposure of macrophage derived monocyte (MDM) to HIV-1 leads to increased IL-8 production, an effect mediated by Tat and the inflammatory cytokine tumor necrosis factor alpha (TNF-α), as well as by gp 120 (Lane *et al.*, 2001).

The aim of the study is basically to evaluate the Serum level of Interleukin-8 (IL-8) and Lymphocyte (CD_4^+ and CD_8^+) numbers in Human Immunodeficiency Virus (HIV) Patients on HAART in Sokoto and controls,to estimate the CD_4^+, CD_8^+ and CD_4^+/CD_8^+ ratio in HIV-positive adults on HAART and controls and to correlate the serum levels of IL-8, CD_4^+ and CD_8^+ T cells count of HIV-positive adults on HAART and controls.

MATERIALS AND METHODS

Study Subjects/Population

A total of sixty (60) participants were recruited for the study, out of which thirty (30) participants are HIV positive patients on HAART, and Thirty (30) sex- and age-matched apparently healthy individuals as controls.

Study design

Blood Samples Collection and Processing

From each participant, a total of five millilitres (5.0ml) of venous blood was collected using a sterile vacutainer blood specimen bottles, holder and needle. Out of the 5ml, three millilitres (3.0 ml) was dispensedinto a sterile plain blood specimen bottle allowed to clot at room temperature and later centrifuged at 3000rpm/min for 5 minutes in order to obtain a clear non-haemolysed serum for the evaluation of interleukin-8 (IL-8); two millilitres (2.0 ml) of the blood specimen was dispensed into a sterile EDTA blood specimen bottle which was used to re-determine and confirmed HIV-status and for the enumeration of CD_4^+ T cell and CD_8^+ T cell count within 3 hours of the blood sample collection.

Ethical Clearance and Informed Consent

The ethical approval for this research was obtained from the Ethics and Research Committee of Specialist Hospital Sokoto. The participants were informed about the study design and only those who gave their consent were recruited for the study.

Measurement of Anthropometric parameters

HIV Screening

The HIV screening was carried out using the WHO screening criteria for developing countries which entails the use of a parallel testing algorithm for serological testing of HIV antibodies in the patient's sera using a combination of three (3) different screening methods, in a stepwise order for the detection of HIV-1 and HIV-2 in the blood: HIV test kits determine, unigold and statpack were used.

Estimation of CD_4^+ and CD_8^+ Cell Counts

The CD_4^+ and CD_8^+T- cells were enumerated using flow cytometry (FCM) method with Cyflow Counter manufactured by Partec, Munster, Germany.

Estimation of CD_4^+ /CD_8^+ Ratio

The CD_4^+ /CD_8^+ Ratio were calculated using the following expression:

CD_4^+ / CD_8^+ Ratio = CD_4^+ cell count (cells/µl)/ CD_8^+ cell count (cells/µl).

Estimation of Interleukin-8 (IL-8)

Serum level of interleukin-8 (IL-8) was measured using quantitative ELISA method, kit was procured from Invitrogen Corporation KHC0081.

Statistical analysis

The data obtained was analyzed using SPSS version 20. The results were expressed as mean ± SEM. Group comparison was made using one-way analysis of variance (ANOVA), paired comparison was carried out using Student's t-test, and P-value of equal to or less than 0.05 ($P \leq 0.05$) was considered as significant.

3. RESULTS

The result in Table1.0 shows socio-economic and demographic characteristics of the study population. The result indicate that 36.7% (11/30) of the HIV subjects are married, 70% (21/30) were women, 56.7% (17/30) of the HIV infected were engaged in one business or the other and the highest educational level attained by these subjects is secondary school 36.7% (11/30).

The result of serum IL-8, CD_4^+,CD_8^+ and CD_4^+/$CD_8^+$of the study subjects presented in Table 2.0 indicate that, serum levels of IL-8 of HIV-patients on HAART (6.78 ± 0.05pg/ml) is significantly higher ($p<0.05$) compared with the corresponding values of controls (6.56 ± 0.09 pg/ml). The CD_4^+ and CD_4^+/CD_8^+ ratio were significantly lower ($p<0.05$) in HIV patients on HAART (424.93 ± 47.61 cells/µland 0.71 ± 0.05 respectively) as compared to the controls (632.63 ± 34.83 cells/µl and 1.10 ± 0.03 respectively). CD_8^+ count in HIV on HAART (555.50 ± 38.73 cells/µl) was almost similar to that of controls (579.98 ± 30.74 cells/µl).

The result of correlation coefficient of IL-8 with CD_4^+,CD_8^+ and CD_4^+/CD_8^+ ratio shown in Table 3.0 indicated that CD_4^+ andCD_8^+ counts correlate negatively with IL-8 in control (-0.064 and -0.074 respectively) and correlate positively in HIV on HAART (0.238 and 0.225 respectively) both of which are statistically insignificant ($p>0.05$) while CD_4^+/CD_8^+ ratio insignificantly correlate with IL-8 in both control and in HIV on HAART ($p>0.05$).

The result of the relationship between anthropometric parameters and immune parameters of Controls shown in table 4.0 indicated that no significant negative correlation of age with CD_4^+ and CD_8^+counts but there was insignificant positive correlation of age with CD_4^+/ CD_8^+ ratio and IL-8. However BMI insignificantly correlated with CD_4^+, CD_8^+ and CD_4^+/ CD_8^+ ratio.

The result in table 5.0 shows the Relationship between Anthropometric Parameters and Immune Parameters in HIV infected Patients on HAART. The BMI is negatively correlated with CD_4^+ and CD_8^+ but positively correlated with CD_4^+/CD_8^+ ratio and IL-8 all of which are statistically insignificant ($p>0.05$). There was a negative correlation of age with IL-8, CD_4^+ and CD_4^+/CD_8^+ ratio but a positive correlation with CD_8^+ all of which are statistically insignificant ($p>0.05$).

The result in table 6.0 indicated that Gender had no influence on serum IL-8 and CD_4^+/CD_8^+ ratio of controls ($p>0.05$). However CD_4^+ and CD_8^+ Counts were influenced by gender ($p<0.05$).

The result in Table 7 indicated that gender had no effect on the immune parameters in HIV infected subjects on HAART ($p>0.05$).

Table 1.0 Socio-Demographic Characteristics of the Study Population

Characteristics	Control (n=30)	HIV on HAART (n=30)
Gender		
Male	20 (66.7%)	9(30%)
Female	10 (33.3%)	21 (70%)
Marital Status		
Single	26 (86.7%)	8 (26.7%)
Married	4 (13.3%)	11 (36.7%)
Widowed	0 (0.00)	6 (20.0%)
Divorced	0 (0.00)	5 (16.7%)
Occupation		
Business	8 (26.7%)	17 (56.7%)
Student	17 (56.7%)	2 (6.7%)
Civil servant	3 (10.0%)	6 (20.0%)
Unemployed	2 (6.7%)	5 (16.7%)
Educational Level		
Primary	1 (3.3%)	3 (10.0%)
Secondary	4 (13.3%)	11 (36.7%)
Tertiary	25 (83.3%)	6 (20.0%)
No formal Education	0 (0.0%)	10 (33.3%)

HIV= human immunodeficiency virus infected patients, HAART= highly active antiretroviral therapy

Table 2.0 Serum IL-8, CD_4^+, CD_8^+ and CD_4^+/CD_8^+ Ratio of the Study Subjects

Parameters	Control (n=30)	HIV on HAART(n=30)	p-value
CD_4^+(Cells/µl)	632.63 ± 34.83	424.93 ± 47.61	0.12
CD_8^+ (Cells/µl)	579.98 ± 30.74	555.50 ± 38.73	0.12
CD_4^+/CD_8^+	1.10 ± 0.03	0.71 ± 0.05	0.007
IL-8 (pg/ml)	6.56 ± 0.09	6.78 ± 0.05	0.003

Values are mean ± SEM; n=number of subjects; CD_4 = cluster of differentiation type 4, CD_8 = cluster of differentiation type 8,IL-8= Interleukin-8.

Table 3: Correlation of serum IL-8 with CD_4^+, CD_8^+ and CD_4^+/CD_8^+ Ratio of the Study Subjects

Parameters	**Control (n=30)**		**HIV on HAART (n=30)**	
	r-value	*p-value*	r-value	*p-value*
CD_4^+(Cells/µl)	-0.064	0.737	0.238	0.204
CD_8^+ (Cells/µl)	-0.074	0.696	0.225	0.231
CD_4^+/CD_8^+	0.062	0.745	0.195	0.303

r = correlation coefficient, n=number of subjects; CD_4 = cluster of differentiation type 4, CD_8 = cluster of differentiation type 8, IL-8= Interleukin-8.

Table: 4. Correlation between Anthropometric Parameters and Immune Parameters of Controls

Anthropometric parameter		CD_4^+(cell/µl)	CD_8^+(cell/µl)	CD_4^+/CD_8^+	IL-8 (pg/ml)
BMI (Kg/m^2)	r-value	0.083	0.076	0.106	0.081
	p-value	0.662	0.689	0.578	0.671
Age (years)	r-value	-0.029	-0.032	0.062	0.089
	p-value	0.879	0.868	0.745	0.064

r = correlation coefficient, n=number of subjects; $CD_{4\ =}$ cluster of differentiation type 4, $CD_{8\ =}$ cluster of differentiation type 8, IL-8= Interleukin-8.

Table 5: Correlation between Anthropometric Parameters and Immune Parameters in HIV on HAART

Anthropometric parameters		CD_4^+(cell/µl)	CD_8^+(cell/µl)	CD_4^+/CD_8^+	IL-8 (pg/ml)
BMI (Kg/m^2)	r-value	-0.043	-0.139	0.101	0.008
	p-value	0.823	0.464	0.595	0.967
Age (years)	r-value	-0.180	-0.055	-0.340	0.223
	p-value	0.341	0.774	0.066	0.237

r = correlation coefficient, n=number of subjects; $CD_{4\ =}$ cluster of differentiation 8,$CD_{8\ =}$ cluster of differentiation 8,IL-8= Interleukin-8.

Table 4.6: Effect of Sex on the Immune Parameters in Controls

Parameters	**Male (n=20)**	**Females (n=10)**	**p-value**
IL-8 (pg/ml)	6.63 ± 0.11	6.42 ± 0.17	0.315
CD_4^+(Cells/µl)	616.45 ± 33.04	665.00 ± 83.32	0.02
CD_8^+ (Cells/µl)	581.38 ± 27.28	577.20 ± 77.39	0.01
CD_4^+/CD_8^+	1.06 ± 0.03	1.18 ± 0.04	0.63

Values are mean ± SEM; n=number of subjects; $CD_{4=}$ cluster of differentiation type 4, $CD_{8=}$ cluster of differentiation type 8, IL-8= Interleukin-8.

Table 4.7: Effect of Gender on the Immune Parameters in HIV on HAART

Parameters	**Male (n=20)**	**Females (n=10)**	**p-value**
IL-8 (pg/ml)	6.75 ± 0.10	6.80 ± 0.06	0.78
CD_4^+(Cells/µl)	503.00 ± 78.93	391.48 ± 58.65	0.61
CD_8^+ (Cells/µl)	622.00 ± 77.72	527.00 ± 44.01	0.68
CD_4^+/CD_8^+	0.79 ± 0.06	0.68 ± 0.06	0.23

Values are mean ± SEM; n=number of subjects; $CD_{4=}$ cluster of differentiation 8,$CD_{8=}$ cluster of differentiation 8,IL-8= Interleukin-8.

DISCUSSION

Interleukin-8 plays an important role in the pathogenesis of HIV (Lane *et al*, 2001). Immune activation as reflected in T cell proliferation is driven by both homeostatic and viral factors that differentially affect the CD_4^+ and CD_8^+ T cell pools. CD_4 T cell proliferation is driven by the homeostatic response to CD_4^+ T cell depletion and by viremia, whereas CD_4^+ T cell proliferation is mainly a result of the levels of HIV viremia. Also demonstrated for the CD_4^+ T cell pool that the homeostatic proliferation induced by CD_4^+ T cell depletion is accelerated by the inflammatory environment generated by the virus (Marta *et al*, 2008).

The current study evaluated the serum IL-8 levels, CD_4^+ and CD_8^+ T lymphocyte numbers in HIV patients on HAART. The study revealed a significant increase in serum IL-8 with CD_4^+ and CD_8^+ decrease in HIV patients on HAART as compared to HIV negative controls. Our results are in agreement with the findings of Marta et al. (2008)who reported that HIV continuously affect CD_4^+ T cells and stimulates macrophages to produce IL-8. Increased serum IL-8levels demonstrated in this study also corroborated with the reports of Boasso et al. (2007) and Paiardini et al. (2005) who independently described Increased serum levels of IL-8 in patients with HIV infection and in other lymphopenic conditions such as post bone marrow transplant and idiopathic CD_4^+lymphopenia.The study conducted by Osunkalu et al. (2015) also showed an increase in plasma IL-8 as compared to HIV negative controls.

The significant increase in serum IL-8 level observed in this study is in agreement with the earlier report by Nikolas et al.(2015) and Kolacinska et al.(2010). This is because macrophages are activated in response to HIV to produce IL-8.The effect of inflammation on the proliferation of CD_4^+ and CD_8^+ T cells is quickly reduced when viremia is suppressed by HAART (Marta *et al.,*2008). After initiation of HAART, the ''rapidly'' proliferating (and dying) CD_4^+ T cell population, is selectively and immediately reduced while there is little effect on the homeostatic forces driving the slowly proliferating T cells (Marta *et al.*, 2008) and (Osunkalu *et al.,*2015). This is confirmed by the present study and this could help to explain why one sees a rapid increase followed by a slow, steady increase in the number of the CD_4^+ T cell pool after the initiation of HAART.

Majority of the HIV positive subjects on HAART were married women aged less than thirty (30) years, this finding is in agreement with the earlier reports in Nigeria (FMOH, 2010), who reported that most of the HIV infected men and women were between the ages of 20 and 29 years. This may be as a result of middle age involvement in economically productive ventures, couple with the quest for physiological satisfaction, which makes it easy for the spread of HIV infection among the middle age group. T cell homeostasis is an important mechanism to assure survival and maintenance of the T cell repertoire through life.

In this study, no correlation exist between CD_4^+ and CD_8^+ counts which is in contrary to the findings of Marta et al.(2008) who revealed a clear inverse correlation between CD_4^+ T cell proliferation and CD_4^+ T cell counts with less of an effect of CD_4^+ T cell counts on the proliferation of the CD_4^+ T cells.In contrast, CD_4^+ T cell proliferation was elevated in all patients with HIV on HAART. The precise pathways leading to CD_4^+ proliferation remain unclear. The study conducted by Doisne et al. (2004) has also shown that CD_4^+ T cells with non-HIV specificities express an activated phenotype in HIV infected individuals, suggesting bystander activation in the setting of HIV infection which is in line with the present study. These data contributed to our understanding of the effects that HIV infection has on the CD_4^+ and CD_8^+ T cell pools. In the case of CD_4^+ T cells one has virus-specific immune responses and inflammatory forces in the presence of homeostatic forces with the net result being activation and slow CD_4^+ T cell depletion. In the case of CD_8^+ T cells, one has virus-specific immune responses and inflammatory forces with the net result being CD_8^+ T cell expansion. Further study of the precise mechanisms involved may help to identify new targets for therapeutic intervention.

However, insignificant positive correlation exist between IL-8 and CD_4^+, CD_8^+ and CD_4^+/CD_8^+ ratio which is in contrary to Ping et al. (2004) report. This controversy is possible because all our subjects are on HAART so that some adverse effects of HIV infection are cancelled.

The Gender appears not to be a factor in the regulation of serum IL-8, CD_4^+, CD_8^+ and CD_4^+/CD_8^+ ratio. This is in agreement with Ping et al. (2004).

CONCLUSIONS AND RECOMMENDATIONS

Serum interleukin-8 significantly increased in HIV patients on treatment with HAART, CD_4^+ and CD_8^+significantly decreased in HIV patients on treatment with HAART, Serum interleukin-8 does not correlate with plasma CD_4^+, CD_8^+ and CD_4^+/ CD_8^+ ratio.

Periodic evaluation of serum interleukin-8 in patients with HIV, to enable professional counseling of patients at high risk of developing Acquired Immunodeficiency Syndrome is suggested and further research should be carried out to elucidate the role of IL-8 in the pathogenesis of HIV/AIDS.

LIMITATION OF THE STUDY

A major limitation of this research work is the small sample size and the time constraint for the conduct of the study. If time permitted, the study would have evaluated the serum IL-8 levels, CD_4^+ and CD_8^+counts on HAART naïve HIV patients.

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