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CD4+T Cells and Tumor Necrosis Factor Alpha in Subjects Living with Human Immunodeficiency Virus in Ekiti State, Nigeria

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Abstract

Human immunodeficiency virus/Acquired immunodeficiency syndrome (HIV/AIDS) is a global pandemic disease with incidence in every country of the world. People living with HIV are prone to developing inflammatory diseases. Thus, the study aimed to assess the level of CD4+ T cells and tumor necrosis factor alpha (TNF- α) in HIV subjects relative to control. A total of 92 subjects (46 HIV positive subjects and 46 apparently healthy subjects without HIV) between the ages of 30-75 years were investigated. The subjects were grouped based on age, antiretroviral therapy and gender. CD4+ T cells were estimated using flow cytometer and TNF- α was estimated using enzyme linked immunosorbent assay. The results obtained showed that there was significant decrease in the levels of CD4+ T cells ($p<0.001$) and TNF- α ($p<0.05$) of HIV subjects compared with apparently healthy subjects (control). The study concluded that the decreased level of CD4+ T cells in HIV seropositive subjects could create an opportunity for opportunistic infections which could cause inflammation and in turn raise the level of TNF- α in the body. Therefore, there is need to monitor these parameters during HIV infection.

Keywords: TNF- α , CD4, HIV, ART

1. Introduction

HIV/AIDS is a global pandemic disease with incidence in every country of the world [1]. It has been shown that approximately 36.9 million people are living with HIV globally and about 940,000 died from AIDS in 2017 [2]. It may be sexually transmitted by contact with or transfer

of blood, pre-ejaculate, semen, and vaginal fluids. Research has shown (for both same-sex and opposite-sex couples) that HIV is not transmissible through sexual intercourse if the HIV-positive partner has a consistently undetectable viral load [3]. Non-sexual transmission can occur from an infected mother to her infant during pregnancy, during childbirth by exposure to her blood or vaginal fluid, and through breast milk [4,5]. Over time, HIV can destroy many cells of the body especially the immune cells such that it cannot fight off infections and other diseases. The damage to the immune system makes it easy for opportunistic infections or cancer to take advantage of the weak immune system thereby making the person to progress to developing AIDS which was first recognized among homosexual men in the United States (US) in 1981 [6,7].

Within a few weeks of HIV infection, the virus begins a massive assault on the gut, which undergoes a significant depletion of memory CD4+ T cells. Such depletion is followed by disruption of the tight junctions in the intestinal epithelium, which may not be fully restored even with early antiretroviral therapy (ART) initiation [8,9]. The persistent inflammation also affects the functionality of the thymus, which is necessary for the achievement of complete immune recovery. In untreated adults, HIV infection causes chronic inflammation and immune activation that induce thymopoiesis, leading to long term thymic dysfunction and clonal exhaustion of T cells, which when persistent can also lead to other infections such as hepatitis, cytomegalovirus, and Epstein-barr virus [10].

Tumor necrosis factor-alpha (TNF- α) is one of the most important proinflammatory cytokines and has been shown to perform diverse functions. It is primarily produced in the peripheral cells including activated monocytes or macrophages [11]. Earlier reports demonstrated that TNF- α stimulated HIV replication in a variety of cells and that its mRNA levels were higher in HIV-seropositive compared to uninfected brain tissue [12,13]. It has also been shown that TNF- α alone or in synergy with other cytokines may upregulate HIV replication and production in host cells [14,15]. Thus, the research was set to assess the level of CD4+ T cells and TNF- α in HIV seropositive subjects in Ekiti State, Nigeria.

2. Materials and Methods

The subjects were recruited from Federal Medical Centre, Ido-Ekiti and its immediate environments in Ekiti State. Ethical clearance was obtained from Federal Medical Center, Ido-Ekiti, Ekiti State. The subjects' consents were sought and obtained before blood specimens were collected from them and voluntary participation was ensured. The subjects included males and females who are HIV seropositive, HIV seropositive subjects who were on ART and apparently healthy subjects which served as control. Individuals who have cardiovascular diseases and all other disease conditions were excluded from this study. The samples collected were used for estimation of CD4 + T cells and TNF- α .

3. Determination of parameters

Estimation of TNF-alpha was carried out using Enzyme linked immunoassay (ELISA) technique [16] while estimation of CD4+ t cells was done using the principle of flow cytometry [17]. The TNF- α ELISA kit (Cat. No: EKHU-0110; LOT No: P20190329h) was purchased from Melsin Medical Co., Ltd., China.

4. Statistical analysis

Results obtained were subjected to statistical analysis using statistical package for social sciences (SPSS) version 23.0, SPSS Inc. Chicago, Illinois, USA. All parameters were expressed as mean ± standard deviation (SD). The student 't' test was the tool of choice in comparing means. Values were statistically significant at $p \leqslant 0.05$ or 0.001 and presented on tables and charts.

5. Results

A total of 92 serum samples from adults between the ages of 30-75 years were analyzed. The samples consisted of 28 (30.43%) cases of HIV subjects on therapy, 18 (19.57%) cases of HIV

subjects not on therapy and 46 (50%) apparently healthy subjects which served as control subjects.

Table 1 shows the mean ± SD of CD4+T cells and TNF- α for the HIV subjects and control groups. There was a significant decrease when CD4+T cells in HIV subjects were compared with control group at $p<0.001$. Table 2 shows the mean ± SD of CD4+T cells and TNF- α of HIV subjects under treatment using different antiretroviral drugs. CD4+T cells and TNF- α did not show significant decrease when HIV subjects using abacavir+zidovudine was compared with those on tenofovir and zidovudine+lamivudine at $p<0.05$.

Figure 1 shows the mean level of CD4+ T cells and TNF- α in HIV positive subject into different age groups. The chart shows that the mean level of CD4+ T cells and TNF- α are higher in HIV subjects within the age group 40-49 and 70-79 than the other age groups.

Figure 2 shows the mean levels of the parameters based on gender The chart showed that both TNF- α and CD4+T cells are both increased in male than in female.

Table 1: CD4+T cells and TNF-α in HIV positive subjects and control subjects

Variables	HIV seropositive subjects N=46	Control N=46	T values	P values
CD4+ T cells (cells/mm^3)	638.22±12.77	798.70±25.71	-5.591	0.001**
TNF alpha (pg/mL)	7.85 ±1.47	13.25±1.42	-2.644	0.010*

**----- significant at $p<0.001$
*----- significant at $p<0.05$

Table 2: CD4+T cells and TNF-α in HIV positive subjects based on drugs used

Variables	Abacavir+ Zidovudine N=9	Tenofovir N=12	Zidovudine+ Lamivudine N=7	F values	P values
CD4+Tcells (cells/mm^3)	690±20.18	693.83±17	681.14±18.59	0.103	0.902
TNF alpha (pg/mL)	13.57±6.50	8.17±1.49	4.23±1.60	1.276	0.297

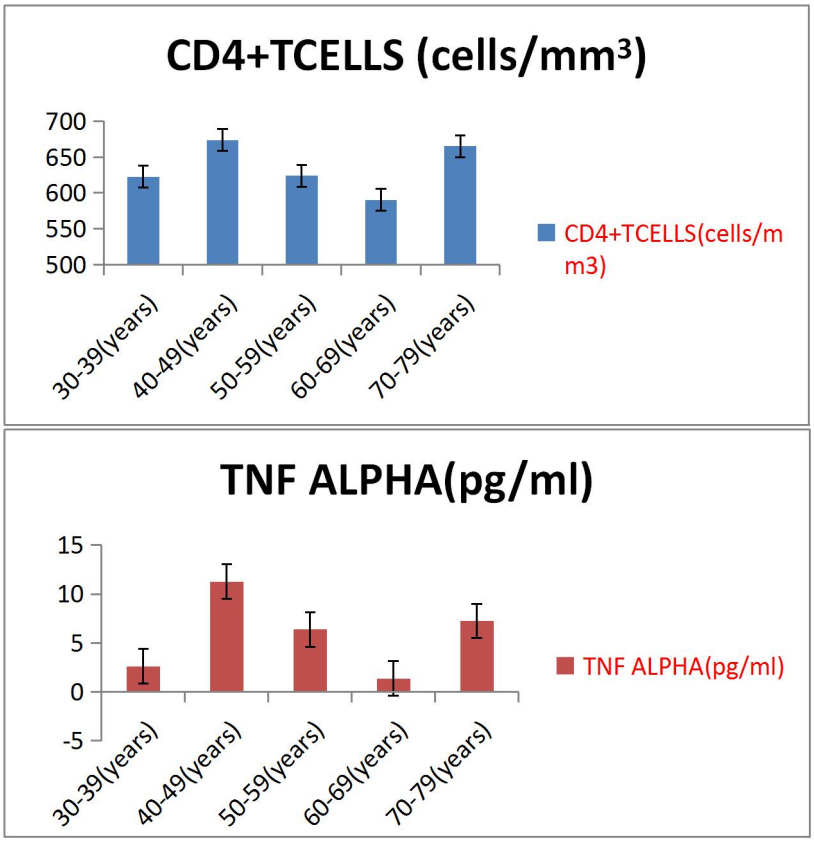

Figure 1: CD4+T cells and TNF alpha in HIV positive subjects according to age group

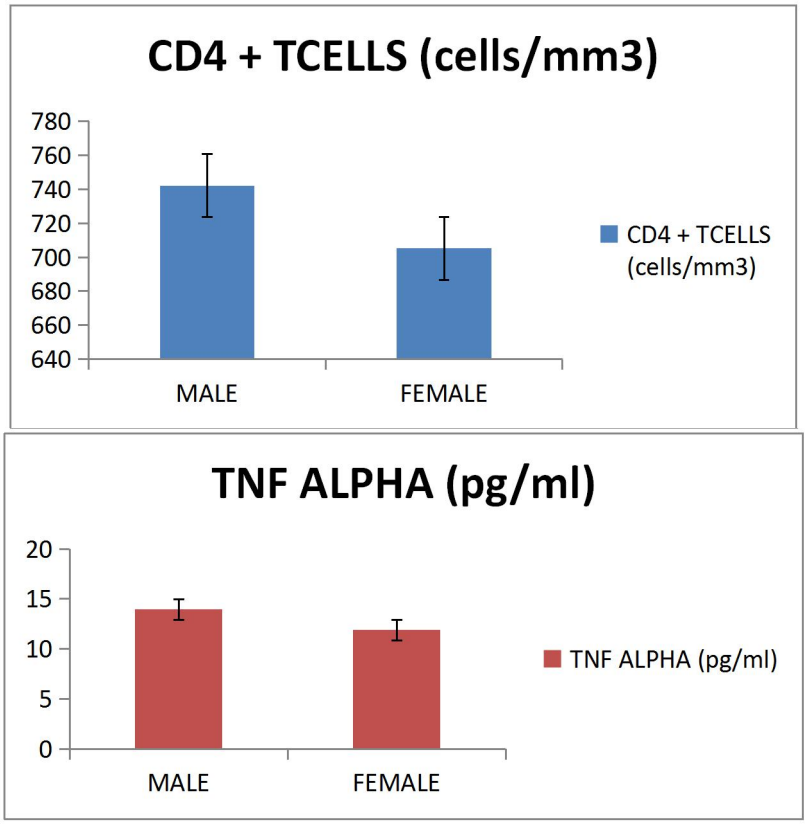

Figure 2: CD4+ T cells and TNF alpha in HIV seropositive subjects according to gender

6. Discussion

In this study there was a significant decrease in CD4+ T Cells in HIV positive patients when compared with control. It has been shown that the level of CD4+T Cells are reduced in the HIV positive subjects as a result of progress in the HIV infection which is in line with other studies which observed that a progression in HIV infection is directly related to the rate at which CD4+ T Cells are lost [18,19]. There was significant decrease in the level of TNF- α in HIV subjects compared with control. The reason for this may be as a result of loss of immunity which was detected in the HIV subjects characterized by decreased CD4+T cells compared with the control. TNF- α and its super family is considered as central mediators of a broad range of biological activities. These activities encompass beneficial effects for the host in inflammation and in protective immune responses against a variety of infectious pathogens [20]. However, other reports showed that TNF- α is associated with severity and progression of HIV related diseases including cognitive impairment and that it could be a potential inflammatory marker for disease monitoring [21, 22].

The level of CD4+T cells and TNF- α according to antiretroviral therapy were also compared. There was no significant difference between tenofovir (TDF) and zidovudine regimen (AZT) in regards to virologic response and this supports the work where it was observed that there was no significant difference between TDF and AZT in regards to virologic response [23]. However, another study observed that tenofovir treatment yielded a higher median increase in CD4 +T cell absolute count compared to zidovudine treatment and immune recovery than zidovudine based highly active antiretroviral therapy (HAART) but equipotent in virologic suppression [24]. The TNF- α did not also show significant difference which supports the study where the drugs used did not affect TNF- α and thus could not cause inflammation [25]. Also, the mean level of CD4+ T cells are seen to be higher in those taking tenofovir regimen than that of zidovudine which supports the study which showed that tenofovir treatment yielded a higher increase in CD4+ T cells compared with zidovudine treatment [24]. However, TNF- α level is not elevated in tenofovir compared with zidovudine regimen which supports the fact that the levels of pro inflammatory cytokines are not affected by ART [26].

There was increased level of CD4+T cells and TNF- α in age group 40-49 and 70-79. The reason may be due to the time each of the age groups commenced the use of drugs. Also, TNF- α may remain unaltered when HIV replication has been well controlled by ART for a long time [27].

CD4+T cells were seen to be higher in male subjects compared with female and this is in agreement with the research done where it was observed that CD4+ T cells in women tend to decrease over time than in men [28].

7. Conclusion

This study showed significant decrease in the levels of CD4+ T cells and TNF- α in HIV positive subjects compared with control. Although the study did not observe significant increase in TNF- α , the study concluded that the decreased level of CD4+ T cells could create an opportunity for opportunistic infections. Presence of these opportunistic infections could cause inflammation and in turn raise the level of TNF- α in the body. Therefore, there is need to monitor these parameters during HIV infection.

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Conflict of interest

The authors declare that there is no conflict of interest whatsoever.

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