ORIGINAL ARTICLE

HAEMATOLOGICAL PARAMETERS, CD4+ T CELLS, PLASMA HIV RNA QUANTIFICATION AND INTERLEUKIN-18 LEVELS IN ADULTS, INFECTED WITH HIV-1 BEFORE AND AFTER INITIATION OF ANTIRETROVIRALTHERAPY IN ZARIA, NIGERIA

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ABSTRACT

Background:
Interleukin (IL)-18, is an important pro-inflammatory cytokine which induces and regulates immune responses in patients with HIV-1 infection. It is reported to be elevated in patients with HIV-1 infection and decreases after highly active anti-retroviral therapy (HAART). However, relatively little is known about its production in HIV infection in the local population.

Aim and Objectives:
To determine and compare the serum levels of IL-18, CD4+ T lymphocytes and HIV viral load quantification following treatment with HAART.

Materials and Methods:
Forty-four (44) HAART naive HIV-1 infected patients undergoing pre-treatment assessment were recruited consecutively at the HIV sub-specialty clinic of Ahmadu Bello University Teaching Hospital Zaria, and commenced on HAART. Haematological parameters were measured using the Sysmex KX-21N automated analyzer, CD4+ T cell counts were quantified using Cyflow counter machine by Partec Germany, IL-18 was assayed using the Quantikine Enzyme Linked Immunosorbent Assay and plasma HIV viral load (pVL) was performed using COBAS Roche Real-Time Polymerase Chain Reaction. All the parameters were assessed at baseline and repeated at 6 months.

Results:
The baseline results versus 6 months were as follows: Haematological parameters were not statistically significant difference following HAART. Serum IL-18 (1345.0 pg/ml vs 741.5 pg/ml; p =0.0188), $CD4^+$ T-cell counts (151.0 cells/µl vs 196.5 cells/µl; p=0.0048) and pVL (6883 copies/ml vs 115 copies/ml, p=0.0001). This shows that while serum IL-18 levels and pVL reduced significantly. $CD4^+$ T-lymphocytes increased with HAART. CD4+ T lymphocytes and IL-18 levels were not significantly correlated to white cell count and pVL following HAART.

Conclusion:
In this study IL-18 levels in HIV-1 infected patients declined following antiretroviral therapy, therefore effective administration of HAART may result in lowering this pro-inflammatory cytokine.

Keywords: HIV-1 infection, serum IL-18, $CD4^+$ T-Cells, plasma HIV viral load, HAART

INTRODUCTION

Human Immunodeficiency Virus (HIV) causes a chronic infection that almost inevitably progresses to acquired immunodeficiency syndrome (AIDS).[1] This viral infection causes immune abnormalities in the infected host, resulting in increased susceptibility to a variety of opportunistic infections.[1] These immune abnormalities occur, at least in part, as a result of the dysfunctional and deregulated expression of several immunologically important cytokines.[2,3] Aberrant production of tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interleukin-12(IL-12), and IL-15 have been well documented in HIV infected/AIDS patients.[3] Optimal production of these cytokines is essential not only for innate host resistance to pathogens but also for the induction, amplification, and maintenance of pathogen-specific immunity. [3] The qualitative nature of the immune response is also determined by the type of cytokines induced in the host, whether they are of T helper 1 (TH1) or of TH2 type.[4] A predominance of TH2-type cytokines has been reported in HIV-infected/AIDS patients and is believed to contribute to the pathogenesis of AIDS. [4] Cytokines induce and or activate transcription factors directly or indirectly, which may regulate HIV-1 replication in human cells IL-18 enhances innate immunity, promotes the development and differentiation of CD4+ naïve T-cells into T-helper 2 type effector cells, regulates TH1- and TH2-type immune responses, and enhances HIV-1 replication. 1 IL-18 is thus recognized as an important regulator of innate and acquired immune responses.[4]

HIV continues to be a major global public health issue, having claimed 36.3 million [27.2–47.8 million] lives so far.[5] There were an estimated 37.7 million [30.2–45.1 million] people living with HIV at the end of 2020, over two thirds of whom (25.4 million) are in the WHO African Region.[5] In 2020, 680 000 [480 000–1.0 million] people died from HIV-related causes and 1.5 million [1.0–2.0 million] people acquired HIV.[5] The Nigeria National HIV/AIDS Indicator and Impact Survey (NAISS) reveals a national HIV prevalence measure of 1.4% among adults aged 15–49 years.[6] Previous estimates had indicated a national HIV prevalence of 2.8%. [6] UNAIDS and the National Agency for the Control of AIDS estimate that there are 1.9 million people living with HIV in Nigeria.[6] The new data differentiate HIV prevalence by state, indicating an epidemic that is having a greater impact in certain areas of the country. The South-South zone of the country has the highest HIV prevalence, at 3.1% in persons aged 15–49 years.[6] HIV prevalence is also high in the North Central zone (2.0%) and in the South East zone (1.9%).[6] HIV prevalence is lower in the South West zone (1.1%), the North East zone (1.1%) and the North West zone (0.6%), but Zaria which is the study area in Kaduna State has a prevalence of 1%. [6].

Haematological abnormalities, particularly the peripheral lymphocytes has been documented as a strong independent predictor of morbidity and mortality in HIV infected individuals.[7] Although it is not part of the criteria for initiating therapy nor used by the WHO for staging HIV, peripheral blood cell abnormalities in an abnormal haemogram are important prognostic tools for morbidity in HIV infection and AIDS.[7]

$CD4^+$ T cells are the principal cellular target of the HIV and their depletion is central to the progression of HIV infections to AIDS.[8] The estimation of peripheral $CD4^+$ T lymphocyte counts is used in taking a decision on initiation of HAART, as a tool for monitoring disease progression and the effectiveness of HAART despite plasma HIV viral quantification being a more sensitive parameter, the latter being much more expensive.[9]

Although the cytokine level decreases in patients treated with HAART, the levels remain above physiologic concentration.[10] Studies have shown that HAART leading to viral suppression also reduced the circulating levels of IL-18.[10] However, there is a rebound elevation of cytokine levels with discontinuation of HAART and with the occurrence of immune reconstitution inflammatory syndromes.[10] IL-18 previously known as the gamma interferon(IFN-γ) inducing factor, was rediscovered as a novel cytokine that plays an important role in promoting TH1 responses by its ability to induce IFN-γ from T and natural killer cells.[3] This pleiotropic

cytokine is produced by activated macrophages, dendritic cells, Kupffer cells, keratinocytes, and enterocytes as well as by the adrenal cortex and neurohypophysis.[3] Induction of IL-18 production is an integral part of the host's innate response to viral pathogens.[4] However, little is known about the expression and regulation of this cytokine in HIV-1 infection in our locality, thus this study is aimed at determining and comparing some haematological parameters, serum levels of interleukin 18 (IL-18), and $CD4^+$ T cells as well as viral load in HIV infected patients before and after 6 months of HAART in Ahmadu Bello University Teaching Hospital (ABUTH) Shika-Zaria, Nigeria.

MATERIALS AND METHODS

Study Area, Study Design and Participant Recruitment

This was a longitudinal study carried out at the Antiretroviral Specialty Treatment and Care Centre at Ahmadu Bellow University Teaching Hospital (ABUTH), Zaria Nigeria. The participants were 44 HIV positive adults (aged ≥ 18 years) selected consecutively (HAART-naïve at baseline) and followed up for six (6) months. Socio-demographic characteristics were obtained using a structured questionnaire.

Consent and Ethical Approval

Ethical approval was obtained from the Health Research Ethic Committee (HREC) of ABUTH, with protocol number ABUTH/HREC/515/2015. Informed and written consent was obtained from each of the participants prior to sample collection. Participants that declined to participate were excluded from the study and this did not affect the care given to such patient/s as all patients received the same standard of care at this specialty clinic regardless.

Specimens Collection and Laboratory Analysis

Fifteen (15) millilitres of venous blood were collected from each participant into K_2EDTA and plain bottles with Aprotinin. All samples were analysed for Complete blood count, $CD4^+$T lymphocyte enumeration and serum IL-18 quantification and HIV RNA plasma viral load (pVL). Samples that could not be tested immediately were preserved at 4°C while samples for pVL and IL-18 were preserved at -80°C until analysed.

Estimation of IL-18

Quantification of IL-18 concentration was carried out by the method of Burtis *et al*.[11] 2006, using CTK Biotech, Inc 4th generation ELISA kit (USA). These assays followed strictly the procedures recommended by the manufacturer, with all samples being tested in duplicate after a 1:10 dilution. The optical density at 450 nm (OD 450) was measured using Synergy HT Auto reader (Bio-Tek Instruments, Inc., Winoosk, VT, USA). Conversion of OD 450 to IL-18 concentration (in pg/ml) was based on a standard curve. The intra- and inter assay coefficients of variation for IL-18 ELISA were 5·0–10·8% and 5·2–10·1%, respectively, which were consistent with results obtained from the tested samples.

Estimation of Absolute $CD4^+$ T Cell Count

The CD4 cell count was conducted using the Cyflow counter machine by Partec Germany. Flow Cytometry is a method by which microparticles in suspension are differentiated and counted according to the cell size, fluorescence emission, and internal structure.[12]

Complete blood count Estimation

The estimation of the full blood count was carried out using the 3-part Sysmex KX-21N automated haematology analyzer.[13]

HIV RNA Viral Load

HIV RNA quantification was performed using COBAS Roche diagnostic USA, Real-Time Polymerase Chain Reaction (PCR) following the standard operative procedures (SOP).[14]

Data Processing/Analysis

Data generated were coded and soft copies kept in a password-protected computer. Data was cleaned and tested for normality. The data were analyzed using IBM *SPSS.* Software (SPSS Inc, Chicago IL) version 17. Student t-test and Wilcoxon matched-pair signed rank test were used to compare quantitative variables. Chi-Square statistics was used to determine statistical association while Spearman's correlation was used for correlation analysis. The level

of significance was set at 95% confidence interval (CI), defined by P ≤ 0.05.

RESULTS

Overall, 44 HIV positive HAART-naïve adults (aged ≥ 18 years) were selected consecutively and studied at baseline and then followed up for six (6) months on HAART. The mean and standard deviation of age of the study subjects was 38.2 ± 9.2 years and there were 30 (68.2%) females and 14 (31.8%) males. The median values of the differential white blood cell parameters at baseline and 6-months after initiation of ART were: Total white blood cells (WBC) with 5.40 (4.05-6.85) x 10^9/Lvs 4.90(3.90 – 7.45) x 10^9/L showing no significant difference (p=0.9690), lymphocytes with 2.00(1.60 – 2.95)x 10^9/Lvs 2.00(1.43– 2.78) x 10^9/L showing no significant difference (p=0.1180), granulocytes with 2.55(1.93 – 3.60)x 10^9/Lvs 2.30(1.80 – 3.33) x 10^9/L showing no significant difference (p=0.4551), Haemoglobin concentration (g/dL)of 11.20 ± 1.62 vs 11.07 ± 2.66 with no significant difference (p=0.7400), and Platelets of 271.9 ± 117.1 vs 244.8 ± 107.5 x 10^9/L with significant difference (p=0.0623),this shows that peripheral blood cells counts are not markedly affected by administration of HAART. IL-18, $CD4^+$T cell, and HIV RNA were compared at baseline and at 6 months using Wilcoxon matched pairs signed rank test as shown in Table 1 below.

Table 1. IL-18 levels, CD4+ T-cell counts and pVL of study participants at baseline and 6 months after HAART

Parameter	Median		P-value
	Baseline	HAART	
IL-18 (pg/mL)	1345(809.8-3082)	741.5(397.8-1575)	0.0188
CD4 (cells/µL)	151.0(70.3-308.0)	196.5(112.5-386.0)	0.0048
HIV RNA (copies/mL)	6883(853.3-60615)	115(20-406.5)	<0.0001

Determined by Wilcoxon matched-pairs signed rank test
IQR: Interquartile range

The result showed that IL-18 decreased from 1345(809.8-3082pg/ml) at baseline to 741.5(397.8-1575pg/ml) at 6 months (p=0.019); HIV RNA decreased from 6883 copies/µl (853.3-60615) to 115copies/µl (20-406.5) at 6months (p=<0.0001), on the other hand, $CD4^+$T cell increased from 151.0 cells/µ/l (70.3-308.0) to 196.50cells/µ/l (112.5-386.0) at 6 months (p=0.0048). This shows that CD4+T cells are reduced, IL-18 levels and pVL are elevated in HAART naïve HIV infected individuals however, administration of HAART results in markedly increased CD4+T cells and a reduction of both IL-18 levels and HIV pVL. Thus Increased IL-18 may play a role in promoting viral replication and disease progression.

Analysis of correlation of IL -18, with WBC, Granulocytes, Lymphocytes, $CD4^+$T cell counts and plasma HIV RNA load of HIV infected patients before and at 6 months of ART determined by Spearman's correlation analysis showed the following: At baseline IL-18 had insignificant negative correlation with lymphocytes (R = -0.2789; p =0.0668; Figure 1) and insignificant positive correlation with WBC, granulocytes, $CD4^+$Tcell counts and HIV RNA load. At 6 months of ART IL-18 had insignificant negative correlations with all the variables, except $CD4^+$Tcell counts (R=0.0093; p=0.549) and pVL (R=0.112; p=0.469), while IL-18 with lymphocytes was significant (R = -0.306; p =0.0437; Figure 2). Therefore, CD4+T cell counts and IL-18 levels cannot be used as an index of determination of HIV pVL.

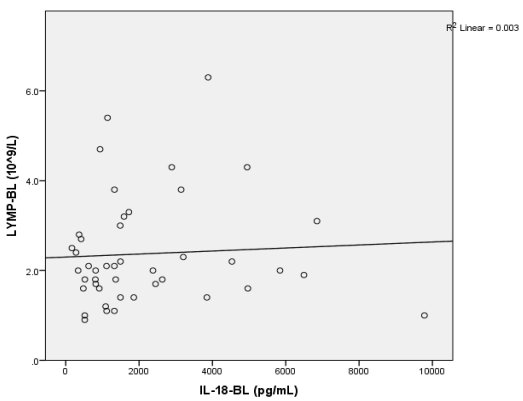

Figure 1: Correlation of IL-18 and Lymphocytes at baseline

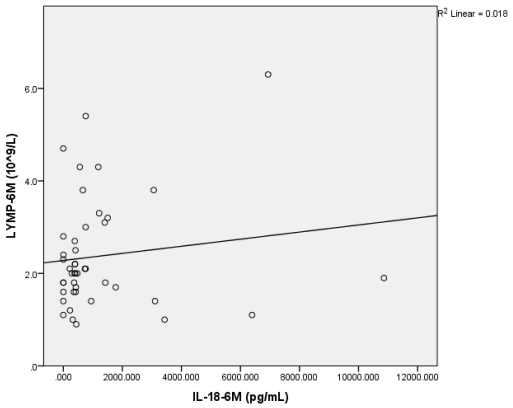

Figure 2: Correlation of IL-18 and Lymphocytes at 6 months (HAART)

DISCUSSION

Human immunodeficiency virus infection remains a major global health issue and several cytokines are released in response to this viral infection. These cytokines have an obvious relevance in redirecting the immune mechanisms and studies have shown that HAART leads to viral suppression as well as to reducing the circulating cytokine levels. In this study, serum IL-18 concentrations in patients receiving HAART decreased significantly compared to baseline levels (Table 2). This observation is consistent with other studies. [15,16,17] Human immunodeficiency virus virions or any of its gene products are potent triggers of inflammasome in circulating monocytes and macrophages, causing the release of IL-18, which primes defence against intracellular pathogens.

HAART has been implicated to turn off inflammasome signalling, which may account for the reduction in IL-18 levels.[18] Earlier studies have reported elevated IL-18 levels in the sera of HAART naïve AIDS patients.[19,20] Similarly, the closely related infection, Simian immunodeficiency virus [SIV] has been shown to stimulate IL-18 production in rhesus macaques.[20] An early stimulation of IL-18 production by these pathogens represents an important defence mechanism of the host meant to enhance both its innate and pathogen-specific adaptive immunities and has been shown to play an important role in controlling these infections.[3] However, several other studies have also shown increased levels of IL-18 in HIV-seronegative subjects with obesity, insulin resistance and type II diabetes.[15] Although HAART is said to reduce IL-18 levels, on the contrary other studies have found that HIV- infected ART initiators, with high levels of IL-18 pre-HAART that failed to normalize during HAART and develop clinical failure than persons who did not have continued high levels of this biomarker.[21] Thus repeated measurements of IL-18, may be useful in identifying HAART-naive persons for whom HAART initiation alone may not be sufficient to prevent HIV complications. Although in our study, we did not repeat IL-18 measurements after 6 months, of interest is the finding of other studies where HAART did not result in normalization of IL-18 levels, even in patients who responded initially to the treatment and had undetectable plasma viral loads.[16] Recent studies also shows that certain anti–HIV-1 drugs (e.g., protease inhibitors) induce secretion of pro-inflammatory cytokines like IL-1b, TNF-α and IL-6. [19] As IL-1b and IL-18 belong to the same family and share similar mechanisms of production, it is highly likely that these anti–HIV-1 drugs also induce production of IL-18 by human macrophages.[19] Thus increased production of IL-18 may promote viral replication and disease progression while a reduction in IL-18 concentration may be an important factor in HAART-related immune restoration, showing IL-18 to have an ambiguous action. Indeed, IL-18 is pro-inflammatory in the presence of IL-12 and anti-inflammatory in the absence of IL-12.

So, IL-12 is needed to prime IL-18 Inflammatory activities and would be an interesting biomarker to assess in further studies involving IL-18.

Findings from this study have shown that although there was a significant increase in CD4+T cell count and decrease in plasma viral load following HAART, correlations of CD4+T cell count and plasma viral load at baseline and during HAART with IL-18 levels recorded no positive correlation.[17] The correlation of $CD4^+$ T-cell counts and IL-18 levels in our study is contrary to the study by Song *et al*., (2006) where serum IL-18 correlates reciprocally with $CD4^+$T-cell counts.[22] The result of this study shows a significant increase in $CD4^+$T-cell counts at 6 months. Higher interleukin-18 levels among patients with low CD4+ T cell counts is consistent with more pronounced inflammation in subjects with more advanced diseases.

Although there was no statistically significant difference in the haemoglobin concentration, total WBC count, granulocytes, and platelets following HAART, the higher WBC observed among the HAART naïve in this study, is in keeping with the higher level of inflammation that is associated with active viral replication and the presence of other opportunistic infections. [23] Additionally, the higher levels of interleukin 18 observed among HAART-naïve patients with relative neutrophilia may also be an indication of ongoing active superimposed opportunistic infections.[20].

Overall, there is no statistical relationship between serum IL-18 levels with either total WBC count, granulocytes and CD4+ T cells count, these findings are highly consistent with those from several earlier studies of treated and untreated patients in populations in Canada and Europe.[16, 17, 18] Similarly, pVL decreased significantly following HAART, however there was no correlation with serum IL-18 levels. The improvement that is associated with the use of HAART as evidenced by both immunological and virological improvement in our patients has been observed by other researchers.[24] This study shows that there is an increase in concentrations of the pro-inflammatory cytokine IL-18 among patients infected with HIV and there is reduction of this cytokine with consumption of HAART. It may be useful to consider serum IL-18 as an informative biomarker for HIV related outcomes. Reduction of IL-18 levels can be an important step in HAART related immune restoration and the biological activities of pro-inflammatory cytokines can reduce the brunt of attack of disease mediated by pro-inflammatory cytokines. Preventing the activity of IL-18 is therefore a sensible clinical strategy as the concept of anti-inflammatory cytokine therapy is expanding.

CONCLUSION

HIV-1 infection is associated with elevated IL-18 production, but declines following antiretroviral therapy, therefore, effective administration of HAART may result in lowering this pro-inflammatory cytokine.

Limitations:
The study did not follow up the patients for longer than 6 months, the type of ART drug regimen is not included and the study did not measure the natural antagonists of IL-18 and other chronic infections such as tuberculosis were not excluded.

Conflict of Interest:
The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions:
BO contributed to the design of the study, data collection, purchase of reagents, conducted the tests and manuscript preparation, whilst BAA designed, analysed the data and contributed to writing the manuscript. BOPM, OOR and BIS Contributed to the design and writing of the manuscript**.**

Acknowledgement:
The authors wish to express their gratitude to the participants that consented & participated in this study. The logistics support given by the staff of the Antiretroviral Therapy laboratory ABUTH Shika-Zaria is highly appreciated.

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