



Original Research Article

Evaluation of Some Inflammatory Markers in Hiv Patients on Antiretroviral Therapy Attending Federal Teaching Hospital, Owerri

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Abstract

Human Immunodeficiency Virus (HIV) is the infectious agent that causes acquired immunodeficiency syndrome (AIDS). The introduction of Antiretroviral Therapy (ART) has revolutionized HIV care, transforming a once-terminal illness into a manageable chronic condition. A case control study to evaluate the levels of inflammatory markers (IL-6, IL-10, C-reactive protein) in HIV patients on Antiretroviral therapy attending Federal Teaching Hospital, Owerri was carried out. A total of 130 subjects aged between 18 and 65 years comprising 50 HIV positive subjects on ART. Also 50 HIV negative subjects and 30 HIV positive subjects not on ART who served as control 1 and 2 respectively were recruited for the study. Seven millilitres (7ml) of their venous blood samples were aseptically collected after an overnight fast of 8-12 hours. ELISA technique was used for the determination of IL-6, IL-10 and CRP. Data were analyzed with student t-test, ANOVA and Pearson correlation using the SPSS version 23. Test with a probability value of $p < 0.05$ was considered statistically significant and the results were expressed as mean \pm standard deviation. Results showed that CRP ($10.21 \pm 1.67 \text{ mg/L}$), IL-6 ($12.96 \pm 2.47 \text{ pg/ml}$) and IL-10 ($6.93 \pm 4.51 \text{ pg/ml}$) were significantly higher ($p < 0.05$) in HIV positive on ART compared with those of HIV negative group ($4.51 \pm 0.97 \text{ mg/L}$, $4.64 \pm 0.96 \text{ pg/ml}$, 4.91 ± 4.68) respectively. CRP ($10.21 \pm 1.67 \text{ mg/L}$) and IL-6 ($12.96 \pm 2.47 \text{ pg/ml}$) were significantly higher ($p = 0.01$) in HIV positive on ART compared with HIV positive not on ART ($7.65 \pm 2.18 \text{ mg/L}$ and $10.35 \pm 4.12 \text{ pg/ml}$) respectively. From these findings, HIV infection and ART significantly influence these inflammatory parameters. Routine measurements of CRP, IL-6, IL-10, should be incorporated into HIV treatment and management protocols to enhance disease monitoring.

Keywords: inflammatory markers, HIV patients, antiretroviral therapy, Owerri.

INTRODUCTION

HIV, or human immunodeficiency virus, is a lentivirus (a type of retrovirus) that causes acquired immunodeficiency syndrome (AIDS). This is a condition in which the immune system slowly fails, allowing dangerous infections and malignancies to grow. The human immunodeficiency virus (HIV) is a virus that targets the immune system. Since they first appeared in the early 1980s, the Human Immunodeficiency Virus (HIV) and acquired immunodeficiency syndrome (AIDS) have been some of the worst public health problems in the world. HIV/AIDS still affects millions of people around the world, putting a lot of stress on healthcare systems, especially in places with few resources [1]. As of 2023, there are about 40 million people around the world living with HIV. AIDS-related diseases have killed millions of people in the last 40 years. Sub-Saharan Africa is still the centre of the pandemic, with about two-thirds of all HIV cases in the world. In this area, women and teenage girls are more at risk because of long-standing gender inequities, socio-economic differences, and cultural norms that make it harder for them to get prevention and treatment services [2]. HIV infection rates have been going up in places other than Sub-Saharan Africa, like Eastern Europe, Central Asia, and portions of Asia. These increases are especially noticeable among groups at high risk, such as persons who inject drugs (PWID), sex workers, men who have sex with men (MSM), and migratory workers [5]. There are still big gaps in access to prevention, diagnosis, and treatment, which makes it harder for the world to respond to the epidemic [3].

Antiretroviral therapy (ART) has changed HIV care in a big way; it has turned a disease that used to be deadly into a chronic condition that can be managed. ART stops viruses from replicating, lowers the risk of transmission, and makes life better for people who with HIV. By the end of 2021, almost 28.7 million of the 38.4 million persons living with HIV around the world were getting ART [4]. Along with ART, prevention methods like handing out condoms, voluntary medical male circumcision, harm reduction initiatives, and pre-exposure prophylaxis (PrEP) have all played a big role in lowering the number of new infections. These interventions have been notably successful in places where everyone can get to them; but, getting everyone to have equal access is still a problem, especially in low-income nations where money and infrastructure are still problems [5]. Even if things are getting better, there are still a lot of problems that make it harder to stop the spread of HIV. Stigma and prejudice against people living with HIV (PLHIV) prevent many from getting tested and treated, which keeps the cycle of undiagnosed and untreated cases going. Marginalised populations, such as MSM, transgender individuals, and PWID, experience social isolation that heightens their susceptibility to HIV infection [6]. Another big problem is that there isn't enough money. Funding for HIV programs around the world has gone down in recent years. This has made people worry about whether ART coverage will last and whether prevention services will grow. New co-infections like tuberculosis and hepatitis make it even harder to treat diseases. The emergence of drug-resistant HIV strains jeopardises the efficacy of existing treatment protocols, hence requiring sustained investment in research and innovation [7]. The Human Immunodeficiency Virus (HIV) has a big impact on the immune system and overall health, making it a major public health problem around the world. The virus mainly attacks the immune system, namely CD4+ T cells, which are important for coordinating immunological responses. HIV infects these cells, makes copies of itself, and eventually kills them, which slowly weakens the immune system [14]. As the infection progresses, the decline in CD4+ T cells undermines the body's capacity to combat opportunistic infections and malignancies, which are hallmark features of acquired immunodeficiency syndrome (AIDS). [8] Chronic immunological activation is one of the first things that happens when a person gets HIV. This syndrome is caused by the virus replicating all the time, microbes moving via weakened mucosal barriers, and immune cells dying and being replaced all the time. Chronic immunological activation not only speeds up the loss of CD4+ T cells, but it also makes other types of immune cells, like macrophages, dendritic cells, and natural killer cells, tired and unable to work properly. This systemic inflammation raises the risk of acquiring health problems that are not related to AIDS, such as heart disease, neurocognitive difficulties, and metabolic syndromes. [9] HIV infection also damages the gut-associated lymphoid tissue (GALT), which is a primary storage place for immune cells. HIV quickly kills off CD4+ T cells in the GALT early in the infection. This makes mucosal immunity weaker and raises the risk of gastrointestinal infections. This damage causes microbes to move around, which makes systemic inflammation and immune system problems worse [10].

HIV infection has effects that go beyond the immune system and affect general health. Without therapy, HIV leads to wasting syndrome, marked by involuntary weight loss and muscle atrophy, which is associated with negative clinical outcomes. The virus also makes people more likely to get other infections, like tuberculosis (TB) and viral hepatitis, which are still major causes of illness and death among people living with HIV (PLHIV). Additionally, HIV infection hastens the progression of these co-infections, establishing a harmful loop of deteriorating health [11]. Antiretroviral therapy (ART) is the most important part of treating HIV. It changes the disease from a deadly one to a chronic condition that can be managed. ART is the use of many antiretroviral medications that work on different parts of the HIV life cycle to stop the virus from replicating and keep the immune system working. ART stops the disease from getting worse by keeping the viral load below detectable levels. It also lowers the risk of opportunistic infections and greatly improves the quality of life for people living with HIV (PLHIV) [12]. One of the main goals of ART is to get the virus under control and keep it there. Effective viral suppression reduces the loss of CD4+ T cells, which are essential for immune system function, hence postponing the onset of AIDS and its accompanying problems. Also, ART lowers systemic inflammation and immunological activation, which are two of the main causes of non-AIDS-related comorbidities such heart disease, metabolic disorders, and neurocognitive loss [13]. The introduction of ART has significantly decreased the transmission of HIV. Research indicates that individuals living with HIV (PLHIV) who maintain undetectable viral loads by consistent antiretroviral therapy (ART) adherence possess an insignificant risk of sexually spreading the virus, encapsulated in the premise "Undetectable = Untransmittable" (U=U). This has changed the way public health works, stressing the fact that therapy may be both a cure and a way to avoid getting sick [14].

Modern ART regimens, which are often called highly active antiretroviral therapy (HAART), are meant to be effective, easy to use, and well-tolerated. Fixed-dose combos that only require one pill a day have made it more easier for people to stick to their treatment and less likely to get tired of it. The introduction of long-acting injectable ART significantly expands treatment alternatives, especially for people encountering difficulties with daily oral medicine [15]. ART is not without its problems, even though it has changed a lot of things. Drug resistance is still a big problem, especially for people who don't always follow their treatment plan or don't have easy access to second-line medicines. Long-term ART use is also linked to side effects such fractures, osteoporosis, kidney and metabolic issues, central nervous system disorders, heart disease, and liver disease. This shows how important it is to keep coming up with new drugs [16]. Fair access to ART is a very important global health issue. ART coverage has grown a lot in the last few years, especially in low- and middle-income countries. However, there are still big differences, and millions of people

can't get life-saving treatment because of money, geography, or institutional problems. To reach the worldwide goal of eliminating the HIV epidemic by 2030, it is important to fill these gaps [17]. It is important to look at biomarkers like interleukins and C-reactive protein (CRP) levels when keeping an eye on HIV patients who are getting antiretroviral therapy (ART). These biomarkers provide significant information regarding immune system functionality, inflammatory status, and general health, facilitating efficient disease management and enhancing treatment efficacy [18]. Interleukins, especially IL-6 and IL-10, are essential for comprehending immunological activation and systemic inflammation in HIV-infected individuals. IL-6 is linked to long-term inflammation and can help predict cardiovascular and other health problems in people who are on ART. High levels of IL-10 often mean that the immune system isn't working properly and may mean that the body isn't able to control the virus even while ART is lowering the virus's levels. Monitoring interleukin levels enables clinicians to detect persistent inflammation and customize therapies to reduce related risks [19].

C-reactive protein is a protein that is used a lot to detect systemic inflammation. Higher CRP levels in HIV patients using ART are associated with an elevated risk of cardiovascular disease and overall mortality. Because ART alone may not completely address chronic immune activation, monitoring CRP assists in identifying residual inflammation and assessing the efficacy of supplementary therapy designed to mitigate inflammatory responses [20]. This investigation is warranted due to the persistent necessity to enhance the health and well-being of individuals living with HIV, especially regarding ART. Assessing biomarkers like interleukins and CRP presents a distinctive opportunity to connect clinical observations with fundamental biological principles. Healthcare providers can devise tailored ways to tackle residual health issues in individuals living with HIV by identifying signs of chronic inflammation, immunological dysregulation, or ART-related toxicities [21]. Previous research has emphasised the significance of biomarkers, such as interleukins and C-reactive protein (CRP) characteristics, in assessing immunological state and disease progression in HIV patients. Nonetheless, there is a scarcity of data about these biomarkers within the Nigerian population, especially among ART-treated patients at healthcare institutions like the Federal Teaching Hospital, Owerri. Moreover, current research insufficiently examines the interaction between these biomarkers and the long-term immunological effects of ART in this context. This gap is important for finding possible additional treatments that could help patients and for making treatments fit the local health and epidemiological characteristics. Filling in these gaps will help us learn more about the immunological status of persons living with HIV on ART in Nigeria and help us find better ways to treat them. This will help the world fight the HIV epidemic more effectively. The results of this study could improve the clinical management of HIV by giving evidence-based advice on how to keep an eye on and improve ART. The project will also help with the larger public health goal of lowering healthcare costs, improving patient outcomes, and lowering the number of non-AIDS-related comorbidities. This research is particularly pertinent for developing strategies to attain equitable and sustainable healthcare solutions, considering the global inequities in ART access and the significant burden of HIV-related illnesses in resource-limited contexts.

MATERIALS AND METHODS

Study Area

The study was carried out at the Heart-to-Heart centre of Federal Teaching Hospital, Owerri Imo State, Nigeria. This is a teaching hospital that takes care of individuals with HIV. Owerri is the capital of Imo State set in the heart of Igbo land. It consists of three Local Government which includes Owerri North, Owerri West and Owerri Municipal. It has an estimated population of about 401,873 according to 2006 census and is approximately 100 square kilometers (40sqm) in area.

Owerri is bordered by Otamiri River to the East and Nwaorie River to the South (NPC, 2006) Owerri has a number of restaurants, fast food centres, hotels, school and market and few industries.

Ethical Consideration, Mobilization and Pre_Survey Contact

Ethical approval was obtained from Heart-to-Heart Clinic at the Federal Teaching Hospital, Owerri

Sample Size Determination

Sample size was determined using the formular

$$n = \frac{Z^2pq}{D^2}$$

n = desired sample size

z = the standard deviation usually set at 95% = 1.96

p = the prevalence of patients with HIV in Imo State

q = 1-p

d = degree of accuracy set at 0.05

According to Caitlin *et al.*, (2022) the prevalence of HIV in Imo State is 1.5%

$$n = \frac{1.962 \times 0.015 \times (1-0.015)}{0.052}$$

$$n = \frac{3.8416 \times 0.034 \times 0.985}{0.002704}$$

$$n = 48$$

The minimum sample size is 48

The maximum sample size that was used for this study is 80.

Study Population

The study population consist of confirmed HIV-positive patients who were currently undergoing Art at the Federal Teaching Hospital, Owerri, HIV positive patients not on ART and also HIV negative subjects.

A total of 130 subjects were recruited for this study, fifty confirmed HIV-positive patients who have been on ART for not less than 6 months, 30 HIV-positive patients who were not yet on ART and fifty apparently healthy participants who served as the control were recruited for this study. The study focused on both male and female subjects aged 18 years to 65years.

Selection Criteria

Inclusion Criteria

The study included;

- i. Confirmed HIV-Positive patients aged between 18 and 65years.
- ii. HIV-Positive subjects who have been on ART for at least 6 months.
- iii. HIV-Negative individuals who served as first control.
- iv. HIV positive patients who were not yet on ART served as second control.
- v. Those who willingly gave their consent to participate in the study.

Exclusion Criteria

The study excluded;

- i. HIV-positive patients aged below 18 and above 65 years.
- ii. Patients who are critically ill or in advanced stage of AIDS.
- iii. Pregnant women or breastfeeding mothers.
- iv. Patient with active co-infections e.g: (Tuberculosis, hepatitis) or other serious infections.
- v. Individuals who are unwilling or unable to provide informed consent.

3.6 Study Design

This study employed a cross- sectional and case control study design utilizing both quantitative and qualitative approaches to evaluate the levels of interleukins, C-reactive protein parameters in 50 HIV patients receiving antiretroviral therapy at the Federal Teaching Hospital, Owerri. This approach allowed for the analysis of the relationship between ART treatment and immune system function, inflammation and overall health status among HIV patients.

Sample Collection

Seven milliliters (7mls) of venous blood was collected aseptically after an overnight fast of 8-12 hours using sterile syringe and needle by a trained phlebotomist. Two (2) mls of the sample was dispensed into a clean plain container and labeled. The sample was allowed to clot after which they were centrifuged at 3,000rpm for 5 minutes to separate and to obtain the serum. The serum was extracted using paster pipette into the appropriate container and stored at -20⁰c prior to use. This sample was used for the determination of interleukins, and C-Reactive protein.

Laboratory assay

All reagents and kits for the work were commercially purchased and the manufacturer's SOPs strictly adhered to.

(A) HIV testing using Determine as described by (Alere, 2013)

Catalogue No MPS5040057: Lot No. 66329K19K100R

(b) UNIGOLD commercial kit as described by Trinity Biotech (2022). Catalogue No: lot No. HIV5110041: Batch No. 1206502

(C) Quantitative Measurement of Interleukin 6 (IL-6) using ELISA Kit By Melsin (CAT. NO: EKHU-0140)

(D) Quantitative Measurement of Interleukin 10 (Il-10) Using ELISA Kit by Melsin (CAT. NO: EKHU-0155)

(E) Quantitative Measurement of C-Reactive Protein (CRP) By a Microplate Immunoenzymometric Chemiluminescence Assay (catalogue no: 3175-300)

Statistical Analysis

All data generated in this study was analyzed using software statistical package for social sciences (SPSS) version 23. Difference in mean values between two groups were assessed using student independent T-test while difference in mean values between three groups was assessed using one-way analysis of variance (ANOVA) and post hoc.

The level of statistical significance was set at $P = 0.05$ (95% confidence interval). Tests with a probability value of $P < 0.05$ were considered statistically significant. Values were expressed as mean + standard deviation (mean \pm S.D).

RESULTS AND ANALYSIS

Table 1: Mean \pm standard deviation Values of CRP, IL-6 and IL-10 in HIV Positive on ART, HIV negative group and HIV positive not on ART.

GROUP	VARIABLES (UNITS)		
	CRP (mg/L)	IL-6 (pg/mL)	IL-10 (pg/mL)
HIV Positive on ART (A) (n = 50)	10.21 \pm 1.67	12.96 \pm 2.47	6.93 \pm 4.51
HIV Negative (B) (n = 50)	4.51 \pm 0.97	4.64 \pm 0.96	4.91 \pm 4.68
HIV Positive not on ART (C) (n = 30)	7.65 \pm 2.18	10.35 \pm 4.12	7.57 \pm 1.23
F-Value	161.903	135.591	8.834
P-Value	*0.001	*0.001	*0.001
A vs B	*0.001	*0.001	*0.004
A vs C	*0.001	*0.001	1.000
B vs C	*0.001	*0.001	*0.001

Key: CRP = C-reactive protein, IL-6 = interleukin 6, IL-10 = interleukin 10, ART = Antiretroviral therapy, mg/L = Milligram per litre, pg/mL = pictogram per milliliter, n = number of samples, vs = versus, ANOVA = Analysis of variance, * = statistically significant at $p < 0.05$.

ANALYSIS

Table 1 shows the mean \pm SD ANOVA and Post-Hoc levels of CRP, IL-6 and IL-10 in HIV positive on ART, HIV negative group and HIV positive not on ART.

There were significant differences in the mean levels of CRP, IL-6 and IL-10 among the various groups ($p < 0.05$).

Moreover, the multiple post-hoc comparison showed that mean \pm SD levels of CRP (10.21 \pm 1.67mg/L), IL-6 (12.96 \pm 2.47pg/ml) and IL-10 (6.93 \pm 4.51pg/ml) were significantly higher ($p = 0.001$, $p = 0.001$, $p = 0.004$) respectively in HIV positive on ART when compared with the mean \pm SD values of CRP (4.51 \pm 0.97mg/dl), IL-6 (4.64 \pm 0.96pg/ml) and IL-10 (4.91 \pm 4.68pg/ml) in HIV negative group.

Also, the mean \pm SD values of CRP (10.21 \pm 1.67mg/L) and IL-6 (12.96 \pm 2.47pg/ml) were significantly higher ($p = 0.01$) in HIV positive on ART when compared with HIV positive not on ART (7.65 \pm 2.18mg/L and 10.35 \pm 4.12pg/mL) respectively.

However, there was no significant difference ($p=1.000$) in the mean \pm SD values of IL-10 (6.93 \pm 4.51pg/ml) in HIV positive on ART when compared with HIV positive not on ART (7.57 \pm 1.23).

Moreso, the mean \pm SD values of CRP (4.51 \pm 0.97mg/L), IL-6 (4.64 \pm 0.96pg/ml) and IL-10 (4.91 \pm 4.68pg/L) were significantly lower ($p=0.001$) in HIV negative group when compared with those of HIV positive not on ART (7.65 \pm 2.18mg/L, 10.35 \pm 4.12pg/mL and 7.57 \pm 1.23pg/ml) respectively.

Table 2: Mean \pm SD values of Inflammatory Parameters in Male versus Female HIV positive individuals on ART.

Variables (Units)	Male (n = 25)	Female (n = 25)	T – value	P – value
CRP (mg/L)	10.06 \pm 1.78	10.37 \pm 1.56	-0.658	0.514
IL-6 (pg/mL)	12.31 \pm 2.12	13.61 \pm 2.66	-1.916	0.061
IL-10 (pg/mL)	6.74 \pm 1.09	7.21 \pm 1.24	-1.148	0.256

Key: CRP = C-reactive protein, IL-6 = Interleukin 6, IL-10 = Interleukin 10, n = number of samples, ART = Antiretroviral therapy, HIV = Human immunodeficiency virus.

Table 2 shows the mean \pm SD levels of CRP, IL-6 and IL-10 in male versus female HIV positive individuals on ART. The mean \pm SD levels of CRP ($10.06 \pm 1.78\text{mg/dL}$), IL-6 ($12.31 \pm 2.12\text{pg/ml}$) and IL -10 ($6.74 \pm 1.09 \text{ pg/mL}$) showed no significant difference ($p = 0.514$, $p = 0.061$, $p = 0.256$) respectively in males when compared with those of female HIV positive individuals on ART ($10.36 \pm 1.56\text{mg/L}$, $13.61 \pm 2.66\text{pg/ml}$ and $7.12 \pm 1.24\text{pg/ml}$) respectively.

DISCUSSION

The research underscores the correlation between HIV status, antiretroviral medication (ART), and specific inflammatory markers, specifically interleukin-6 and interleukin-10. The findings from this investigation, as presented in Table 4.2, indicate that the mean values of CRP, IL-6, and IL-10 were significantly elevated in individuals with HIV who were undergoing antiretroviral therapy (ART) compared to the mean values of these parameters in the HIV-negative group. This may be attributable to the fact that HIV infection induces chronic immunological activation and inflammation, even among those undergoing ART, and patients with HIV frequently demonstrate immune dysregulation that persists despite ART. Conversely, HIV-negative patients generally have reduced levels of these biomarkers, indicating a more equilibrated immune response [22]. When HIV infects a person, their immune system makes cytokines, immunoglobulins, and immune cells [23]. C-reactive Protein (CRP) is a protein that the body makes when it is inflamed and might show how active the immune system is. High CRP levels ($>10\text{mg/L}$) are linked to more inflammation and the possibility of treatment resistance. HIV infection causes chronic inflammation, which raises CRP levels. ART can lower inflammation, but CRP levels may still be higher than those of people who don't have HIV [24]. IL-6 is a pro-inflammatory cytokine that encourages inflammation. HIV infection elevates IL-6 levels, hence exacerbating chronic inflammation [25]. IL-10 is a cytokine that controls the immune response and fights inflammation. HIV infection disturbs the equilibrium between pro-inflammatory and anti-inflammatory cytokines, resulting in elevated IL-10 levels. High levels of IL-10 frequently mean that the immune system isn't working properly and may show that the body isn't able to regulate the virus even while ART is lowering the virus's levels [26]. Furthermore, the concentrations of CRP and IL-6 were markedly elevated in HIV-positive individuals undergoing ART compared to those not receiving ART. These results are in agreement with previous research that indicates sustained elevations of CRP and IL-6 in this study signify chronic inflammation, possibly attributable to microbial translocation and residual immunological dysregulation. HIV assaults the immune system, while antiretroviral therapy (ART) aids in regulating HIV replication, hence facilitating the immune system's recovery, including antibody generation [27, 28]. These trends align with the findings of [29, 30], which indicated that increased inflammation in HIV patients frequently associates with anaemia, thrombocytopenia, and immunological dysfunction.

CONCLUSION

This study showed that people with HIV had higher levels of IL-6, IL-10, and CRP in their blood than healthy controls. The examination of interleukins (6 and 10) and C-reactive protein levels in HIV patients undergoing ART yields significant insights into their health status, facilitates clinical decision-making, and enhances the comprehension of HIV management in Nigeria. Consequently, these parameters should be regarded as essential markers for the treatment, management, and monitoring of HIV-positive individuals on ART.

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