

Evaluation of Serum Cytokines Biomarkers in HIV-Infected Subjects Before and After Commencement of Haart in Federal Teaching Hospital Gombe

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Abstract

The Human Immunodeficiency Virus (HIV) infection causes the acquired immunodeficiency syndrome (AIDS), which is a systemic disorder characterized by severe impairment and progressive damage of both cellular and humoral immune responses. The aim of this study is to evaluate the effect of long administration of HAART on haematological indices, immunological biomarkers, Biochemical and some cytokines in HIV infected subjects at federal teaching hospital Gombe. This is a cross sectional study carried out from May to December 2021 on 132 HIV infected adults. The design entails collecting pre and post blood sample after treatment with highly anti-retroviral treatment (HAART) for more than six months. Out of the 132 subjects studied, 66 were HIV sero-positive and these were the test group, while the remaining 66 were HIV Sero-negative drawn from client visiting blood bank of FTH Gombe to make voluntary donation and HIV Testing Unit (HTS) for Marital and Medical Certificate of Fitness Screening and served as control group. All the subjects were between the ages of 15 - 65 years and are aged matched with the control group. Demographic data was collected using structured questionnaire. The protocol entails collecting 9mls of venous blood from those who were seropositive for HIV 1 &2 before commencement of HAART. This sample is designed pre- treatment sample and these individuals were followed up for 6 months and a second sample collection was done some were lost to follow up and so the attrition was compensated by also collecting blood samples from other HIV individual who had been on HAART for several years. 4ml was dispensed into EDTA bottle for Full blood count using ELIZA analyzer. $P < 0.05$ was considered as significant. The result of the analysis indicated that the mean $\pm$ se of Interleukines-2 (IL-2) (pg/ml) and Interleukines -6 (IL-6) (pg/ml) parameters for the control group are 65.43 ± 3.37 and 34.74 ± 1.20 respectively. The mean $\pm$ se of the treatment naïve for IL-2 and IL-6 are 101.61 ± 16.47 and 14.63 ± 1.65 respectively. Also, the mean $\pm$ se of the treatment/HAART for Interleukines-2 (IL-2) (pg/ml) and Interleukines -6 (IL-6) (pg/ml) are 67.43 ± 1.40 and 10.80 ± 0.45 respectively. The IL-2 significantly higher among treatment Naïve HIV infected participants when compared with treatment and control group with (p-value < 0.05). The result is also shown in table 2.0 Above. From this work it is clear that all the cases in this regard, confirm the fact that Long-term HAART remarkably drops the viral load and inflammation in HIV positive subjects and shows that a low-level inflammation continues which probably leads to chronic inflammatory state, morbidity and

mortality in this group of subjects among HIV positive patients in this part of Northern Nigeria despite the reported side effect

INTRODUCTION

An estimated 36.7 million people worldwide are living with Human Immunodeficiency Virus (HIV) with 1.8 million new infections in 2016 [6]. In Sub-Saharan Africa, more than 23.5 million people are living with HIV. Current records show that 20.9 million people living with HIV are accessing antiretroviral therapy (ART), which is the current effective management of HIV [7]. Modern drug discovery has thus transformed HIV into a manageable chronic infectious disease [13]. Human immunodeficiency virus is the causative agent of AIDS. Since 1981, when the first AIDS cases were reported, more than 30 million people have been diagnosed as infected with HIV [17]. A large part of the infected individuals lives in relatively poor and developing countries. Due to the large scale of morbidity and mortality it causes, HIV is fast becoming a major threat in developing third world countries including the Indian sub-continent [12]. Infection with HIV is associated with prolonged latent period during which the virus continues to actively replicate, usually resulting in symptomatic illness [9]. The HIV disease progression, which is highly variable in infected individuals, is characterized as rapid, typical, or intermediate and late or non-progressors. Most infected individuals (70-80%) experience intermediate disease progression in which they show HIV RNA rise, CD4 T-cell decline and later development of AIDS related illness in 6 - 10 years. 10-15% of the infected patients whose CD4+T-cells rapidly decline and go into AIDS within few years of infection are called rapid progressors. The late progressors (5%) can remain asymptomatic and healthy without showing significant changes in CD4 T-cell Counts even for 10 years [6]. HIV is known to be associated with a wide range of immunological and haematological changes. The immunological changes include depletion in CD4+ T cell, cytokine dysregulation and immune dysfunction. The dominant immunologic feature of HIV infection is progressive depletion of the helper T cell (CD4+ T cell), which reverses the normal CD4:CD8 ratio and subsequently lead to immunodeficiency. CD4+ T cells interact with antigen presenting cells (APCs), B cells, cytotoxic T cells (CD8+ T cells) as well as natural killer cells. Thus, infection and depletion of CD4+ T cell population could induce profound immunodeficiency in such patients [4].

MATERIAL AND METHOD

Study Area

This research was conducted in Federal Teaching Hospital Gombe (FTHG), a tertiary health care institution and referral facility in Gombe State.

Study Design

This is a cross sectional study, involving the collection of pre and post treatment sample in individuals that tested positive to HIV antibodies with HAART in Federal Teaching Hospital Gombe (FTHG), a tertiary health care institution in Gombe. Individuals who were seropositive to HIV1 or HIV2 at HIV testing (HTS) unit and are yet to be enrolled into HAART treatment plan were randomly selected and enrolled into the study. They were designated HAART naïve. Venous blood sample was collected before commencement of HAART. This sample was designated treatment naïve. Sample was also collected from those who had been on HAART from six months upwards the control group and designated control. Those who were enrolled at pretreatment phase were followed up and samples equally collected after six upwards.

Sample collected at > six months was designed post treatment (long term). Demographic data were also collected using a structured questionnaire.

Study Subjects

One hundred and thirty-two (132) subjects were recruited into this study, male and female of 15 years and above. Sixty-Six (66) of the subjects were HIV Sero-negative clients, 41 Females and Males 25, serving as Negative Control. The remaining Sixty-Six (66) were HIV infected subjects 36 Females and 30 males and have history of no previous antiretroviral therapy. The written consent of the clients will be sought for before the recruitment. Ethical approval will be acquired from Ethical and Research Committee of FTH Gombe before commencement of the study.

Ethical Considerations

Ethical approval was acquired from the Gombe State Ethical and Research Committee and FTHG Ethical Committee before commencement of the study. Written informed consent was obtained from the clients prior to the commencement of the study.

Statistical Analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) version 21.0. Frequencies and percentages were calculated. ANOVA was used to compare the mean Variants. The result was presented as mean $\pm$ standard error of mean. A p- value of ≤ 0.05 was considered as significant in all statistical comparisons.

Sample Collection

A volume of 4 ml of blood drawn from the participants was dispensed into a plain Vacuum tube and clear serum was harvested. Samples were transferred immediately to the laboratory and serum was stored at -80 °C until analyzed for serum cytokines.

CYTOKINES QUANTIFICATION

Interleukin 2 and 6 (IL-2, IL-6) (Nanjing Pars Biochem co., Ltd. China).

The human IL-6 ELISA from Abcam (ab46053) is used for the in vitro quantitative Determination of interleukin 2 in human serum, plasma, buffered solutions or cell Culture medium. The assay recognizes both natural and recombinant human IL-6.

3.9.6.1 Principle

The IL-kit is a solid phase sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Interleukins-6 and 2 was coated onto the wells of the microtiter strips provided. During the first incubation, Interleukines-6 present in the sample or standard and a monoclonal anti-interleukins- 6 and 2 antibodies conjugated to biotin are simultaneously incubated. Following incubation, unbound biotinylated anti-Interleukin-6 is removed during a wash step. Streptavidin-HRP is added and binds to the biotinylated anti-Interleukin-6 and 2. After incubation and a wash step a substrate solution reactive with HRP is added to wells. A coloured product is formed in proportion to the amount of interleukin-6 and 2 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm.

3.9.6.1 Assay protocol

All reagents were mixed gently. After 5 minutes of reconstituting the stock standard with the standard diluent, serial dilution of the standards was made (1000, 500, 250, 125, 62.5, 31.25, and 0). The controls were also reconstituted as recommended. 100µl of the serially diluted standards will be pipetted into appropriate micro wells (0 -1000 in A-H, A being the blank). 100µl of samples and control was pipette into the sample and control wells. The biotinylated anti-IL-6 was prepared as Recommended and 50µl was pipette into all wells. The plate was covered with Plates cover and incubated for 1 hour at room temperature. After the incubation, the plate was washed 3 times. The streptavidin-HRP solution was prepared and 100µl was dispensed into all the wells. The plate was covered and incubated at room Temperature for 30 minutes. The wells were washed 3 times. 100µl of the ready-to -use chromogen TMB substrate solution was added into all the wells and will Incubated in the dark for 15 minutes. The enzyme -substrate reaction was stopped by adding 100µl of the H2SO4 stop reagent into each well and was read at 450nm.

RESULT

Demographic Characteristics of Studied Participants

The study enlisted 132 participants of which 66 were control group selected from client visiting blood bank unit of FTH Gombe to make voluntary donation and HIV Testing Unit (HTS). Another 66 participants were recruited from the HIV clinic at FTH Gombe served as treatment naïve and six months after commencement of HAART. The mean±sem age (15- 65Yrs) of the HIV patients are (28.88±0.822) for those on treatment/HAART and (30.53±1.239) for treatment naïve. The mean age for the control group was (29.06±0.741). The male and female participants for control group were 26 and 40 respectively and the male and female participants for both treatment naïve and treatment/HAART group were 29 and 37 respectively.

Table 1.1: Demographic characteristic of the study subjects

Variables	HIV/AIDs (Before and After Treatment) (n = 66)	HIV/AIDs (Negative controls) (n = 66)
Gender		
Female	33 (55%)	38 (63.3%)
Male	27 (45%)	22 (36.7%)
Age (Years)		
Mean age	30.535 ± 1.239	29.067 ± 0.741

Key: n= number sample; %= percentage

The result of the analysis indicated that the mean±se of Interleukines-2 (IL-2) (pg/ml) and Interleukines -6 (IL-6) (pg/ml) parameters for the control group are 65.43 ± 3.37 and 34.74 ± 1.20 respectively. The mean±se of the treatment naïve for IL-2 and IL-6 are 101.61 ± 16.47 and 14.63 ± 1.65 respectively.

Also, the mean±se of the treatment/HAART for Interleukines-2 (IL-2) (pg/ml) and Interleukines -6 (IL-6) (pg/ml) are 67.43 ± 1.40 and 10.80 ± 0.45 respectively. The IL-2 significantly higher among treatment Naïve HIV infected participants when compared with treatment and control group with (p-value < 0.05). The result is also shown in table 2.0 below.

Table 2.0: Comparison of Cytokines Parameters among HIV-/Control, HIV+/Naïve and HIV+/HAART Subject

Parameters	(Control) (Mean ± Se)	(Naïve) (Mean±se)	(HAART) (Mean ± Se)	F-Test	P Value
IL-2 (pg/ml)	65.43 ± 3.37	101.61 ± 16.47	67.43 ± 1.40	4.36	0.014*
IL-6 (pg/ml)	34.74 ± 1.20	14.63 ± 1.65	10.80 ± 0.45	11.35	0.000*

KEY: *= Percentages, IL-2= Interleukines-2, IL-6 (pg/ml) = Interleukines-6

DISCUSSION

The aim of this study is to evaluate the effect of long-term administration of HAART on some serum cytokines biomarkers among HIV/AIDs reactive clients on antiretroviral therapy on a long term from 6 months compared with HIV/AIDs negative (control) and sero positive case not on treatment.

The Mean±Sem age of the HIV subjects are (28.88±0.822) for Long-term on HAART and (30.53±1.239) for Pre-Treatment. The Mean±Sem age of the control group was (29.06±0.741). The male and female participants for control group are 22 and 38 respectively and the male and female participants for both Pre-treatment and Long-term on HAART group were 26 and 34 respectively.

HIV infections are of major public health importance worldwide and especially in developing countries, where it affects the socio-economic status of individuals, families, communities, and the entire societies [1]. Antiretroviral drug treatment for HIV infections has considerably improved the prognosis for HIV infections by repairing immune veracity and regulating opportunistic infections [10]. The introduction of HAART, which is an amalgamation therapy, has helped in suppressing human immunodeficiency viral replication to undetectable levels and sustaining this suppression for months or years in a substantial number of persons. However, there have been some downsides to HAART treatment including the possible upsurge in cytopenias and biochemical abnormalities in treated patients.

In this study, we sought to determine the and predict the degree of immunological changes of HIV infected patients that may be associated with the administration of HAART.

The mean±sem of cytokines parameters (IL2 (pg/ml) and IL6 (pg/ml) for control, long-term on HAART, and pre-treatment are 65.43 ± 3.37, 101.61 ± 16.47 and 67.43 ± 1.40 and 34.74 ± 1.20, 14.63 ± 1.65 and 10.80 ± 0.45 respectively with all p-value< 0.05. This indicates that high HIV viral load may be the main driver of high plasma IL-2 levels, which significantly reduce upon effective treatment with long-term antiretroviral therapy as observed in the study. A study conducted by [2] found minimal effects of HAART on the levels of cytokines after 16 to 24 weeks of treatment. Some other reports did not observe any influence of HAART on levels of IL-6 while other reports observed a decrease of IL-6 after initiating HAART.[3], found similar results in serum levels of IL-6, which decreased after 6 months of HAART,

CONCLUSION AND RECOMMENDATIONS

From this work it is clear that all the cases in this regard, confirm the fact that Long-term HAART remarkably drops the viral load and inflammation in HIV positive subjects and shows that a low-level inflammation continues which probably leads to chronic inflammatory state,

morbidity and mortality in this group of subjects. We therefore recommend regular routine assessment of those cytokines parameters at every follow-up visit and early initiation and adherence to HAART on every eligible HIV-positive patient.

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