ORIGINAL ARTICLE

Can serum concentration of C-reactive protein, albumin and body weight serve as an index of disease progression and treatment assessment in HIV/AIDS subjects?

Adeola O **OLUBOYO**[1]
Uchenna N **ONYEKACHI**[1]
Charles C **ONYENEKWE**[1]
Bernard O **OLUBOYO**[2]
Rebecca C **CHUKWUANUKWU**[1]
Chukwujekwu NC **ODIEGWU**[1]
George O **CHUKWUMA**[1]
Uchenna F **ONWUASOANYA**[1]
Anulika O **ONYEMELUKWE**[1]

[1]*Department of Medical Laboratory Science Faculty of Health Sciences and Technology College of Health Sciences Nnamdi Azikiwe University Nnewi Campus Anambra State, NIGERIA*
[2]*Institute of Human Virology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, NIGERIA*

Author for Correspondence
Adeola O **OLUBOYO**
Department of Medical Laboratory Science
Faculty of Health Sciences and Technology
College of Health Sciences
Nnamdi Azikiwe University
Nnewi Campus
Anambra State, NIGERIA

E-mail: adeolaboyo@yahoo.com
Phone: +234-805-416-8105

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ABSTRACT

Background: Human immunodeficiency virus infection induces an acute phase response which is marked by changes in the plasma concentrations of acute phase proteins and a fall in CD4+T-cell counts and body weight.
Objective: To determine whether serum concentration of C-reactive protein, serum albumin level and body weight can serve as an index of disease progression and treatment assessment in HIV/AIDS.
Methodology: The study investigated 80 subjects (40 subjects on anti-retroviral therapy and 40 not on therapy) and 40 sero-negative subjects (control) attending the HIV/AIDS Clinic in Nnamdi Azikiwe Teaching Hospital, Nnewi, Nigeria. We determined serum concentrations of C-reactive protein, serum albumin levels and body weights. The C-reactive protein was estimated using semi-quantitative method, albumin level was estimated using bromocresol green method, and biuret method was used for total protein. The CD4+T-cell count of the subjects was determined using CyFlow Analyzer while their weights were measured using high precision weighing balance.
Results: We observed that the mean weight (Kg), CD4+T-cells count (count/mm^3), serum albumin (g/L), and total protein (g/L) of HIV subjects on therapy and those not on therapy were significantly lower ($p<0.05$) than in the control subjects. The mean C-reactive protein (mg/dL) was significantly higher in subjects on therapy compared to those not on therapies and the control subjects, $p<0.05$.
Conclusion: In resource-poor regions or remote areas or villages where CD4+T-cell counts and viral load tests may not be available, the concentration of C-reactive protein, serum albumin level and changes in weight may serve as a reliable alternative marker for disease progression and treatment assessment for HIV subjects.

Keywords: *Acute-phase proteins, anti-retroviral therapy, total protein*

INTRODUCTION

Human immunodeficiency virus (HIV), the causative agent of Acquired Immune Deficiency Syndrome (AIDS) is a global pandemic causing the greatest public health concern. HIV infection is one of the most common lethal infections worldwide.[1] It is a disease of the human immune system and it

progressively reduces the effectiveness of the immune system and leaves the individuals susceptible to opportunistic infections and tumours.[1] Furthermore, acute and chronic infection induce an acute phase response, which is marked by changes in the plasma concentrations of a number of plasma proteins and lipids.[2]

According to Bryan, the acute-phase proteins (C-reactive protein and albumin) are a sensitive marker of inflammation and tissue damage.[3] Moreover, small elevations in C-reactive protein (CRP) concentrations have been shown to indicate increased risk for cardiovascular disease and possibly colon cancer.[4] Serum albumin levels are a good predictor of the severity of HIV disease in individuals who are not taking anti-retroviral therapy and can also indicate the extent of a patient's response to HIV treatment.[5] Although albumin levels are not a marker for HIV infection states, they have been found to be a strong predictor of mortality in HIV-positive adults and children.[5] Low serum albumin levels is a marker for malnutrition, may reflect poor nutritional status at early stages of HIV disease before changes in body weight or other clinical matters are apparent.[6]

The hallmark of human immunodeficiency virus (HIV) infection is the cytopathic effect on the CD4+ bearing cells (helper T4 cells).[7] Apart from these cells, other CD4+ bearing cells such as macrophages, B-lymphocytes, microglial cells, haemopoeitic stem cells, rectal mucosal cells, *Kupffer* cells and liver sinusoid epithelial cells are also affected.[8] The lower the CD4 cell count, the more likely there is progression of the disease.[9] The use of anti-retroviral treatment reduces both the mortality and morbidity of HIV infection, but these drugs are expensive and access to these medications is not easy in most countries of the developing world.[9]

METHODOLOGY

Study Area

This research was conducted in the HIV Clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, South-East Nigeria.

Research Population

The research population included the HIV sero-positive patients visiting the HIV clinic in NAUTH Nnewi from April to June 2010, and also HIV sero-negative age-matched and sex- matched subjects who served as controls.

Sample Size and Sampling Procedure

The sample population comprised of one hundred and twenty subjects (120) aged 20-65 years. Forty subjects were on anti-retroviral therapy and forty subjects were not on anti-retroviral therapy. They were all attending the HIV/AIDS Clinic of the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. The remaining forty subjects comprised apparently healthy students and staff of NAUTH and they served as controls. After obtaining the consent from the subjects, 5mL of venous blood was drawn aseptically into dry plain containers. The serum was separated immediately after clot retraction and stored at -20^{0}C for a period of 2weeks until the time of assay. The CRP was determined using the CRP Latex semi-quantitative reagent test kit manufactured by Teco Diagnostics. The CD4+Tcell counts of the subjects were determined using Cyflow Analyzer. Also, their weight was measured using high precision weighing balance. The total protein was estimated using Biuret reagent and albumin levels was estimated using Bromocresol green kits manufactured by Randox Ltd, United Kingdom.

Statistical Analysis: The statistical method used is the Analysis of Variance (ANOVA). The statistical analysis was carried out using SSPS version 17.0 with significance level of 0.05.

RESULTS

The results were expressed as mean ± standard deviation and presented in tables 1 and 2. Table 1 shows the mean ± standard deviation of CD4+Tcell count (284.4±172.7, 414.7±307.2, 811.2±224.9), weight (66.63±13.6, 81.7±105.8, 82.9±104.6), CRP (82.9±104.6, 2.61±2.84, 1.24±1.09), albumin (39.0±10.4,

36.6±8.13, 50.4±7.06) and total protein (66.5±14.94, 63.4±15.26, 76.5±4.91) in HIV Subjects on drug therapy, those not on drug therapy and control subjects, respectively. There were significant decreases ($p<0.05$) in the mean weight, CD4+Tcell count, albumin and total protein when the HIV sero-positive subjects were compared with control subjects but, there was a significant increase in the mean CRP when the HIV sero-positive subjects were compared with control subjects.

Table 1. CD4+Tcell count, weight, CRP, albumin and total protein in HIV subjects on drug therapy, those not on drug therapy and control subjects

Parameters	HIV Subjects on drug therapy n= 40	HIV Subjects not on drug therapy n=40	Control subjects n= 40	F-value	p-value
Weight (Kg)	66.63±13.6	81.7±105.8	82.9±104.6	0.733	0.030*
CD4+Tcell counts /mm³	284.4±172.7	414.7±307.2	811.2±224.9	41.97	0.000*
CRP (mg/dL)	4.76±7.31	2.61±2.84	1.24±1.09	6.033	0.003*
ALBUMIN (g/L)	39.0±10.4	36.6±8.13	50.4±7.06	12.763	0.000*
TOTAL PROTEIN (g/L)	66.5±14.94	63.4±15.26	76.5±4.91	11.666	0.000*

* =*Significant at p<0.05*

Table 2. CD4+T- cells count, weight, C-reactive protein, albumin and total protein in subjects based on different drug combinations

Drug combinations	Weight (kg)	CD4 + cells count /mm3	CRP (mg/dL)	Albumin (g/L)	Total Protein (g/L)
Combivir and Nevirapine **(n=19)**	67.0±12.4	303±195.7	5.39±7.62	30.6±5.124	67.1±16.4
Nevirapine and Tenofivir **(n=4)**	72.5±19.1	266.5±68.9	2.99±2.49	33.0±2.94	60.8±6.65
Combivir and Emitricitabine **(n=6)**	65.8±18.2	241.2±146.0	1.87±1.09	32.8±4.71	72.8±11.51
Lamivudine, Stavudine and Nevirapine **(n=2)**	53.5±30.4	202.5±188.8	13.6±17.0	35.0±1.41	66.0±11.3
Emitricitabine and Nevirapine **(n=1)**	72.0±0.00	528.0±0.00	3.20±0.00	32.0±0.00	57.5±26.0
Zidovudine, Emitricitabine and Tenofivir **(n=4)**	66.8±7.68	286.0±175.4	7.05±12.0	29.0±8.76	57.5±26.0
Lamivudine, Zidovudine and Emitircitabin **(n=6)**	65.3±4.79	255.5±208.3	1.58±1.16	31.0±5.38	66.5±15.0
F-value	0.431	0.492	0.892	0.682	0.513
p-value	0.853	0.810	0.512	0.666	0.794

Table 2 shows the levels of CD4+Tcell count, weight, C-reactive protein, albumin and total

protein in HIV sero-positive subjects on different drug combinations (Combivir and Nevirapine; Nevirapine and Tenofivir; Combivir and Emitricitabine; Lamivudine, Stavudine and Nevirapine; Emitricitabine and Nevirapine; Zidovudine, Emitricitabine and Tenofivir; Lamivudine, Zidovudine and Emitircitabine**).**

There was no statistically significant difference (*p>0.05*) when the CD4+Tcells count (303±195.7; 266.5±68.9; 241.2±146.0; 202.5±188.8; 528.0±0.00; 286.0±175.4; 255.5±208.3), weight (67.0±12.4; 72.5±19.1; 65.8±18.2; 53.5±30.4; 72.0±0.00; 66.8±7.68; 65.3±4.79), CRP (5.39±7.62; 2.99±2.49; 1.87±1.09; 13.6±17.0; 3.20±0.00; 7.05±12.0; 1.58±1.16), albumin (30.6±5.124; 33.0±2.94; 32.8±4.71; 35.0±1.41; 32.0±0.00; 29.0±8.76; 31.0±5.38) and total protein (67.1±16.4; 60.8±6.65; 72.8±11.51; 66.0±11.3; 57.5±26.0; 57.5±26.0; 66.5±15.0) were compared based on the different drug combinations.

DISCUSSION

The use of highly active anti-retroviral therapy (HAART) has clearly resulted in an impressive decline in AIDS-defining illnesses, including wasting.[10] This study revealed that the there was a significant difference (*p<0.05*) in the mean weight when compared in the subjects. Our findings are in line with the study which reported that the control subjects had the highest mean weight compared to those subjects at different stages of HIV infection.[11] This can be explained from the study which reported that majority of men with HIV/AIDS weighed less than 90% of their ideal body weight or had lost more than 10% of their usual weight (compared to the control subjects who are HIV sero-negative).[12]

We also observed that the control subjects had the highest mean CD4+Tcell count. This supports the studies in which the control subjects had the highest CD4+Tcell count compared to subjects at various stages of HIV infection.[11] This is a sign that the immune system of HIV sero-positive subjects has become weakened and the lower the CD4+Tcell count, the more likely there is progression of the disease.[9]

Also, the weights and CD4+Tcell count of subjects on different drug combinations revealed that subjects on nevirapine and tenofivir combinations had the highest mean weight, and those on emitricitabine and nevirapine drug combinations had the highest CD4+Tcell counts. Furthermore, it has been reported that patients taking zidovudine or lamivudine or tenofovir drug combination experienced significantly poor increase in CD4+Tcell counts.[9]

We observed in our study that a high percentage of the subjects on therapy were on combivir and nevirapine drug combination. Moreover, we observed that CRP was elevated among the subjects on anti-retroviral therapy; this may be due to a steady inflammatory state in the subjects as they had the lowest CD4+Tcell count.[13] However, two longitudinal studies have suggested that antiretroviral therapy may significantly increase CRP concentrations despite expected immunologic and virologic responses.[14,15] They indicated that an inflammatory state may persist after the introduction of anti-retroviral therapy and may continue to increase the risk of HIV-associated morbidity.

It was found that there was an increase in mean values of CRP and corresponding decrease in CD4+Tcell counts of the subjects on therapy. Higher CRP concentrations have been associated with lower CD4+Tcell counts and higher HIV RNA levels among HIV-infected individuals because CRP is a component of the innate immune system and may activate the complement system during infections.[14,16]

Although CRP could simply be a marker for opportunistic infections or underlying disease, high circulating concentrations may contribute to promoting disease progression and increasing mortality among HIV-infected patients.

There was an increase in serum albumin concentration in subjects using lamivudine,

stavudine and nevirapine drug combinations and also, there was an increase in total protein levels in subjects using combivir and emitricitabine drug combinations. This finding was similar to a study which showed a rise in albumin levels in subjects receiving non-nucleoside reverse transcriptase inhibitors (NNRTIs) compared to those receiving protease inhibitors (PIs), whereas the albumin levels were lower in those receiving PIs + NNRTI.[17] There was a statistically significant difference when the mean albumin levels of the various groups was compared ($p<0.05$). Also, the plasma concentration of albumin was higher in subjects on therapy compared with subjects not on therapy.

It has been shown that anti-retroviral therapy increases albumin levels compared to subjects who are not on anti-retroviral therapy. Our findings are in line with the study that revealed that there exist significant positive correlations between increase in serum albumin and duration of treatment.[5] Decrease in plasma concentration of albumin in HIV sero-positive subjects may be due to increased trans-capillary escape and catabolic rate promoted by infection.[18] Reduced levels of serum albumin, a major blood protein, is associated with increased mortality in individuals who have certain chronic conditions (e.g. HIV/AIDS).[6] Low serum albumin, a marker for malnutrition, may reflect poor nutritional status at early stages of HIV disease before changes in body weight or other clinical markers become apparent. Thus, monitoring serum albumin could be useful in the clinical setting.

In patients with moderate immune damage (CD4+T-cell count between 350 and 500 cells/mm3), serum albumin <40mg/L could suggest that HAART should be initiated earlier. Similarly, a fall in albumin over time could indicate that a patient already taking HAART should change therapy.[6] There was a significant decrease in the mean total protein levels of subjects on therapy and those not on therapy when compared with the control subjects.

CONCLUSION

We conclude that serum C-reactive protein, albumin levels and weight loss may predict disease progression and treatment assessment in HIV/AIDS patients. Also, in resource-poor regions or remote areas/villages where CD4+T-cell counts and viral load tests may not be available, C-reactive protein, serum albumin level and changes in weight may serve as a reliable alternative marker for disease progression and treatment assessment for HIV subjects.

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