Academic Journal of Life Sciences
ISSN(e): 2415-2137, ISSN(p): 2415-5217
Vol. 5, Issue. 2, pp: 10-14, 2019
URL: https://arpgweb.com/journal/journal/18
DOI: https://doi.org/10.32861/ajls.52.10.14

Academic Research Publishing Group

Original Research **Open Access**

Protein Status and CD4+ Cell Count in HIV Patients on Highly Active Anti-Retroviral Therapy

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Abstract

Background of Study: Malnutrition is associated with repeated opportunistic infections, rapid disease progression, and an increase in the incidence of human immunodeficiency virus (HIV) related mortality. The ability of anti-retroviral therapy (ART) in boosting the immune system depends on the nutritional status of the HIV patient. Aim: The study aimed at investigating the protein status and CD4+ cell counts in HIV patients taking highly active ART. Materials and Methods: The case-control study comprising of a total of 80 participants, compared the protein status and CD4+ cell count among baseline (ART-naïve n=20), switch (ART-resistant n=20), ART follow-up (n=20) patients, and apparently healthy controls (n=20). Results: The total protein of baseline patients was significantly ($P<0.01$) higher than that of the switch, follow-up, and controls. The CD4+ cell count of baseline patients was significantly ($P=0.000$) low compared to follow-up patients and controls. Total protein level and CD4+ cell count of switch patients were significantly ($P=0.000$) lower than that of follow-up patients and controls. Total protein of follow-up patients was significantly ($P<0.02$) higher than that of controls, while the CD4+ cell count of follow-up patients was significantly ($P=0.000$) lower than that of controls. Conclusion: The present study observed low protein along with low CD4+ cell count in switch patients, while a good outcome was observed in follow up patients.

Keywords: Protein; Human immunodeficiency; Cluster of differentiation 4; Anti-retroviral therapy

1. Introduction

Plasma total proteins is primarily composed of 45-55% albumin synthesized in the liver [1], and the remaining fraction, globulins (immune globulins, enzymes, carrier proteins and complement) produced by the liver and the immune system [2]. Plasma proteins have been measured as biochemical markers of nutritional status and in the assessment of protein-energy malnutrition in the general population [3]. Protein Energy Malnutrition (PEM) is a range of pathological conditions arising from a concurrent lack in varying proportions of proteins and calories, commonly associated with infection [4].

In human immunodeficiency virus (HIV) patients, serum total proteins have been previously measured as a biochemical marker of nutritional status [1, 5]. For the purpose of our study malnutrition is viewed in the perspective of undernutrition. In undernutrition, macro- and/or micronutrient supply are continuously below minimum dietary requirements, which lead to diminished function [6, 7]. Before the HIV epidemics, malnutrition was the first cause of immunosuppression worldwide [8] In PEM, cell-mediated and innate immunity [9] are particularly lessened, thereby increasing the risk of viral infection (including HIV) and disease progression [10-12]. Countries where food insecurity and malnutrition are common are also stricken by high HIV incidence and prevalence rates [13, 14]. HIV is a cause of nutritional deficiencies through its effect on nutrient absorption, decreased nutrient intake, and increased energy expenditure creating coexistence between malnutrition and HIV in a vicious perpetuating cycle [15-17]. Malnutrition is associated with repeated opportunistic infections, rapid disease progression, and an increase in the incidence of HIV-related mortality [18]. Undernutrition and weight loss are prevalent amongst highly active antiretroviral therapy (HAART)-treated patients in low-middle income countries which contribute to disease progression and excess early mortality in HIV patients [19]. The HAART regimen is comprised of nucleoside-analog reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and entry inhibitors [20].

The ability of ART in boosting the immune system depends on the nutritional status of the HIV patient. Cluster of differentiation 4 (CD4+) lymphocytes are the primary target cells for HIV [21]. Antiretroviral therapy increases CD4+ cell counts, reduce the risk of opportunistic infections with the ultimate improvement of survival of the HIV infected patient [22]. In patients with HIV, there is an association between low albumin levels, and CD4 count <200 cells/mm^3 [22]. In Nigeria, there were more than 300,000 patients on ART in 2016 and ART has improved the survival of patients with HIV and the quality of life [23].

The aim of this study is to investigate the protein status and CD4+ cell counts in HIV/AIDs patients under HAART in Makurdi, Nigeria.

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2. Materials and Methods

2.1. Study Area

The study involved HIV patients attending the Federal Medical Centre (FMC) Makurdi, Nigeria. FMC is located in Makurdi, the capital city of Benue state, Northcentral Nigeria. It is a tertiary health care institution and the largest hospital in Benue State with a very high patient load. It renders health care services to over 2 million people. Since FMC is a government hospital people of low socioeconomic status come for treatment in this hospital. The hospital provides many health care services including HIV testing, counseling and administration of ART.

2.2. Study Participants

An institutional ethical clearance and informed patients consent were obtained. A total of 80 participants aged 20 to 60 years comprising of 40 males and 40 females were recruited from HIV patients attending FMC Makurdi from April to July 2017. Apparently, healthy controls were sampled from patients attending general health check-up at the hospital within the same period.

2.3. Study Design

The case-control study compared the protein status and CD4+ cell count among four groups; baseline HIV patients who were about to begin ART or ART-naïve patients (n=20), switch HIV patients who were resistant to HAART prompting a subsequent change of drug regimen (n=20), follow-up HIV patients who strictly adhered to their ART regime and responded favorably to their drugs (n=20), apparently healthy controls sero-negative to HIV (n=20).

2.4. Laboratory Methods

Four milliliters of venous blood samples were separately collected, aseptically into ethylene diamine tetraacetic acid (EDTA) and plain vacutainer tubes for the respective determination of CD4+ lymphocytes and total protein from each participant. The CD4+ cell count was determined by flow cytometry using a Partec cyflow machine (Counter 2 model and SL/3). Total protein was analyzed with Hitachi automated chemistry analyzer (902 model). All laboratory analyses adhered to standard operating procedures in FMC laboratory which is nationally accredited. The normal value taken for total protein was 65-85 g/l in both sexes while the cut-off value of CD4 count defining AIDS in HIV patients, according to Nigerian ministry of health recommendation was 200 cells/mm3 (lower than 200 cells/mm^3).

2.5. Statistical Methods

Data were presented as means and standard deviations for continuous variables. Analysis of variance was used for between-group assessments followed by least significant difference *post hoc* significant difference test. All statistical analyses were performed using the IBM Armonk, NY, USA, SPSS version 21. A two-sided $P<0.05$ was considered statistically significant.

3. Results

The total protein and CD4+ cell count in the study participants are presented in table 1. A significant change was observed in mean total protein level and CD4+ cell count among the groups compared. A detailed result by a post hoc analysis presented in table 2 shows the changes in protein and CD4+ cells between the groups compared. Total protein of baseline patients was significantly ($P<0.01$) higher than that of the switch, follow-up, and controls. The CD4+ cell count of baseline patients was significantly ($P=0.000$) low compared to follow-up patients and controls, while a non-significant ($P>0.05$) change in CD4+ cell count was observed between the baseline and switch patients. Total protein level and CD4+ cell count of switch patients were significantly ($P=0.000$) lower than that of follow-up patients and controls. Total protein of follow-up patients was significantly ($P<0.02$) higher than that of controls, while the CD4+ cell count of follow-up patients was significantly ($P=0.000$) lower than that of controls.

Table-1. Total protein and CD4+ cells in the study groups

Parameter	Baseline (n=20)	Switch (n=20)	Follow-up (n=20)	Control (n=20)	F-value	P-value
T. Protein	96.17±9.94	66.04±9.58	88.71±8.26	81.64±6.21	44.43	0.000*
CD4+ cells	156.80±152.55	100.80±57.23	434.80±252.47	812.20±281.19	50.00	0.000*

*significant, total protein-T. Protein, cluster of differentiation 4 (CD4+) cells

Table-2. Post-hoc test amongst the groups studied

Patients	T. Protein	P-value	CD4+ cells	P-value
Baseline	96.17±9.94		156.80±152.55	
Switch	66.04±9.58		100.80±57.23	
		0.000*		0.392
Baseline	96.17±9.94		156.80±152.55	
Follow-up	88.71±8.26		434.80±252.47	
		0.008*		0.000*
Baseline	96.17±9.94		156.80±152.55	
Control	81.64±6.21		812.20±281.19	

		0.000*		0.000*
Switch	66.04±9.58		100.80±57.23	
Follow-up	88.71±8.26		434.80±252.47	
		0.000*		0.000*
Switch	66.04±9.58		100.80±57.23	
Control	81.64±6.21		812.20±281.19	
		0.000*		0.000*
Follow-up	88.71±8.26		434.80±252.47	
Control	81.64±6.21		812.20±281.19	
		0.011*		0.000*

*significant, total protein -T. Protein, cluster of differentiation 4 (CD4+) cells

4. Discussion

Over 850 million people worldwide and 200 million adults in Sub-Saharan Africa suffer from Malnutrition [24, 25]. Countries most affected by HIV are also stricken by elevated rates of food insecurity and malnutrition [19] HIV infection and insufficient nutritional intake are part of a vicious cycle that contributes to immunodeficiency and negative health outcomes. However, the effect of the overlap between HIV infection and under-nutrition on the immune response following antiretroviral initiation remains unclear.

The present study observed increases in serum total protein in the baseline (ART-naive patients), follow-up patients (good treatment outcome) and a decrease in switch patients (ART-resistant patients) compared to HIV negative healthy controls. A decrease in CD4+ cell count, reflecting poor immunity was observed in the baseline and switch patients while normal levels of CD4+ cell count, reflecting improved immunity was found in the follow-up patients. In the switch patients, serum total proteins decreased along with CD4+ cell count. The marked increase in total protein levels of baseline patients is in line with the studies of Serpa et al., Audu et al., Patil & Raghuwanshi who reported elevated proteins in untreated HIV patients [1, 26, 27].

This finding could be explained by the positive impact of hypergammaglobulinemia and other globulin fractions on plasma protein level in ART-naïve HIV patients. Chronic HIV infection induces hypergammaglobulinemia via polyclonal B-cell activation and spontaneous secretion of immunoglobulins by abnormally activated B-cells [28]. Other globulin fractions are also abnormally elevated among untreated HIV-infected patients due to the expression of the aberrant immune activation [29-31]. However, globulin levels decrease in HIV patients who achieve controlled viremia after starting HAART, normalizing serum total protein levels. This is due to improved hypergammaglobulinemia and B-cell dysfunction [1].

The present study observed no association between low CD4+ cell count and protein status in baseline and follow-up patients. In populations from developing countries, research has failed to demonstrate an association between low CD4+ counts and markers of undernutrition amongst newly diagnosed seropositive HIV individuals compared to seronegative controls [32, 33]. The present study is also in line with the study of Feleke et al., who did not observe any significant association between CD4+ cell count and the malnutrition indicators in 395 follow-up HIV patients [5]. Their result was ascribed to the excellent adherence of patients to HAART and the effectiveness of HAART in limiting HIV disease progression [5]. The normal total protein observed in the follow-up patients may be due to the effectiveness of HAART on HIV patients. This is supported by reports linking increased availability of HAART to the improvement of the nutritional status of patients in developing countries [34-37].

The present study observed reduced total protein levels along with low CD4+ cell count in the switch patients. A decrease in serum total protein in HIV infection has been associated with protein-losing enteropathy, Hypercatabolism, malabsorption, reduction of intake due to loss of appetite, fatigue, depression and side effects of medications [38, 39]. HIV is a cause of nutritional deficiencies through its effect on (1) nutrient absorption; HIV-mediated inflammation and induced mucosal immune cell damage in the gastrointestinal tract leads to diarrhea and malabsorption, (2) decreased intake; odynophagia induced by esophageal candidiasis (an opportunistic infection) which limits the capacity to feed oneself, (3) increased energy expenditure; due to HIV association with opportunistic infections [17, 18].

Highly active antiretroviral therapy (HAART) leads to increased requirements for macro- and micronutrients, high metabolic demands [40] and low appetite [41] which perpetuate undernutrition [42]. Malnutrition exacerbates side effects [43-46], alters drug pharmacokinetics [47], and impinges on adherence [48] thereby limiting the beneficial effects of the therapy. The concomitant low CD4+ cells along with low total protein levels observed in the switch group may be explained by poor immune reconstitution secondary to deficient nutritional status. Due to dwindling funding of HIV programs in developing countries, there is need to optimize the therapeutic success of first-line ART in order to delay the need for a therapeutic switch, and limit rates of resistance, which are on the rise, in these regions [34].

5. Conclusion

The present study observed low protein along with low CD4+ cell count in switch patients, while a good nutritional outcome was observed in follow up patients. Despite theoretical and experimental evidence linking malnutrition and altered immune function, there exists no consensus concerning the impact of malnutrition on the immune response in HIV patients receiving HAART. The Nutritional status of HIV patients and HIV disease progression association should be thoroughly investigated for developing strategies to diminish the mortality of HIV patients on HAART.

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