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Levels of Immunoglobulin Classes Are Not Associated with Severity of HIV Infection in Nigerian Patients

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ABSTRACT

The serum concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), total protein and albumin were measured in 35 Human Immunodeficiency Virus—positive, HAART (highly active antiretroviral therapy) naïve subjects attending the PEPFAR (President's Emergency Plan for AIDS relief) clinic, University College Hospital, Ibadan and in 30 apparently healthy control subjects to assess the relationship between serum protein, immunoglobulin concentrations and laboratory indices of HIV disease (CD4 cell counts and viral load). Serum IgG (1008.6 ± 530.7 mg/dL), IgA (170.4 ± 69 mg/dL) and total protein (9.9 ± 1.7 g/dL) levels were higher among HIV positive subjects compared with mean values in healthy subjects (549.8 ± 193.8 mg/dL, 106.8 ± 26.4 mg/dL and 7.8 ± 0.5 g/dL respectively). The median serum IgM concentration (131 mg/dl) was significantly higher in HIV positive subjects compared with 35 mg/dL in healthy controls ($p < 0.001$). Mean serum albumin concentration was significantly lower among HIV positive subjects (3.7 ± 0.7 g/dL), compared with 4.3 ± 0.3 g/dL in healthy subjects ($p < 0.001$). There were no significant differences observed in the levels of the immunoglobulin classes when HIV subjects with CD4 counts of <200 cell/µL were compared with subjects with CD4 counts $\geq$ 200 cells/µL. There was also no statistically significant correlation observed between viral load and serum immunoglobulin levels.

Keywords: HIV; Immunoglobulins

1. Introduction

The Human Immunodeficiency Virus—Acquired Immunodeficiency Syndrome (HIV-AIDS) pandemic continues to be one of the leading causes of infectious disease morbidity and mortality worldwide especially in sub-Saharan Africa. Although the sub-continent accounts for ten percent (10%) of the world's population, seventy percent (70%) of the 40 million persons who were living with AIDS at the end of 2001 were in Sub-Saharan Africa [1].

HIV infection results in a gradually progressive disease with multisystem involvement, multiple opportunistic infections and many types of cancers. HIV infection also has serious consequences on the immune system of the body with a resultant progressive immunodeficiency that leaves the body vulnerable to pathogens and the development of malignancies [1]. This underscores the need for an array of laboratory technical innovations for the diagnosis and monitoring of the infection.

Plasma viral load and CD4$^+$ T cell counts in conjunction with the patient's clinical status are presently the gold standards of assessing and monitoring the clinical progression in HIV infection [2]. The routine use of these parameters in developing countries is limited by high cost, availability of required technology and trained personnel.

Other surrogate markers have been used for monitoring the progression of HIV infection and assessing response to therapy. These include lymphocyte phenotypic markers like CD38, HLA-DR, IL-2R, CD45RO and markers of apoptosis such as Fas [3]. A large number of soluble markers of immune activation have also been evaluated as prognostic indicators in HIV infection. These include serum/plasma levels of neopterin, β2-microglobulin, tumour necrosis factor alpha (TNFα), soluble CD8$^+$ and other soluble cytokine receptors [3]. However a common factor in the use of these markers is the cost involved in performing these assays which unfortunately, would set them beyond the reach of poorer nations.

In the light of the foregoing, it is desirable to identify other surrogate markers of disease progression that are relatively inexpensive and technically simple which

could help the clinician to effectively monitor the course of infection and decide when to initiate therapy [4]. This would be especially welcome in developing countries where health budgets are less robust. Arinola *et al.* (2005) [5,6] from Nigeria and Lyamuya *et al.* (1999, 1994) [7,8] from Tanzania have previously demonstrated elevated serum immunoglobulin levels in HIV seropositive individuals. Furthermore, a steady rise in IgA with progression to AIDS has been demonstrated in different cohorts of patients [4,9]. These studies did not relate immunoglobulin levels with viral loads. This study was aimed at measuring serum levels of immunoglobulin subclasses in relation to CD4+ severity grouping of HIV-positive, HAART-naive individuals to assess the potential of immunoglobulin classes in the prognosis and monitoring of HIV infection.

2. Materials and Methods

This study was approved by the Joint UI/UCH Ethical Committee.

2.1. Subjects

A comparative study was carried out between September 2008 and November 2008 on thirty five (35) newly diagnosed HIV positive patients, who were not on antiretroviral therapy. The patients were recruited into the study at the PEPFAR clinic based at the University College Hospital, Ibadan, Nigeria. Only asymptomatic patients with a confirmed diagnosis of HIV infection were included. Pregnant female patients were excluded from the study. Blood samples were taken for serum immunoglobulin quantitation, total protein and albumin after informed consent was granted. Control samples were obtained from thirty (30) healthy adult HIV-negative blood donors at the blood bank of the University College Hospital and volunteer staff. Demographic data was obtained from each subject.

2.2. Laboratory Analysis

Sample collection: 5 ml of venous blood was drawn from superficial veins of the antecubital fossa with minimal trauma. The blood samples were dispensed into sterile bottles without additives for the assay of total serum protein, albumin and immunoglobulins (IgG, IgA, IgM). Blood was centrifuged to obtain serum which was separated and stored at −70˚C until the time of assay.

CD4+ cell counts: Peripheral blood mononuclear cells obtained from heparinized fresh blood samples of subjects were labeled with fluorescent anti-CD3, anti-CD4, or anti-CD8 specific monoclonal antibodies and analyzed on a Cyflow (Partec©, Germany) flow cytometer at the Virology laboratory of the UCH Ibadan to obtain absolute CD4 cell counts.

HIV viral load: HIV RNA levels in subject plasma samples were quantified using the *AMPLICOR HIV*-1 *MONITOR TEST* version 1.5 (Roche©). This is based on polymerase chain reaction technology to achieve maximum sensitivity for the quantitative detection of HIV RNA in anticoagulated plasma.

Serum total protein and albumin: Serum total protein and albumin were assayed using a Roche© Hitachi 902 auto-analyser which operates based on spectrophotometric principles.

Serum Immunoglobulins: Immunoglobulin classes were quantified using immunoplates based on the anti-genantibody precipitation reaction in agar gel.

2.3. Statistical Analysis

This was carried out using the SPSS statistical software version 16. *Descriptive statistics* were generated for variables; mean and standard deviation for normally distributed quantitative variables while median and range were generated for skewed quantitative variables.

Inferential Statistics: Student t-test was used to compare the mean of normally distributed variables while Mann-Whitney test was used to compare the median of skewed quantitative variables. Correlation testing was carried out using the Pearson's correlation test. All statistical tests were two-tailed at the 5% probability level.

3. Results

A total of 35 HIV positive individuals were recruited into this study. These consisted of 12 male and 23 female subjects with a mean age of 31 years. There were 30 control subjects including 13 males and 17 females. The total study population consisted of 65 individuals and the results are presented below (see **Tables 1-3**).

4. Discussion

It is noteworthy that several investigators have estimated serum immunoglobulin profile of HIV-positive patients in different countries [4,7-10]. In this study, the serum immunoglobulin profile of HIV sero-positive individuals attending the PEPFAR Clinic, UCH was measured.

Patients were aged between 22 years to 42 years with a mean age of 31 years. Twelve of these were male while 23 were females. This may not reflects the true sex prevalence given the greater inclination of women to attend health facilities than men in this environment. The present study was hospital-based. The UNAIDS 2008 report [11] indicates that the percentage of women among people living with HIV has remained stable (at 50%) for several years.

The findings in this study showed that the mean serum levels of IgG, IgA, IgM and total protein in HIV- positive person were significantly elevated (see **Table 1**).

Table 1. Serum concentrations of immunoglobulins and proteins in study population.

Subjects	n	IgG* (mg/dL)	IgA* (mg/dL)	Tot. protein* (g/dL)	Albumin* (g/dL)		IgM** (mg/dL)
HIV negative	30	549.8 (193.8)	106.8 (26.4)	7.8 (0.5)	4.3 (0.3)		35 (52)
HIV positive	35	1008.6 (530.7)	170.4 (69.)	9.9 (1.7)	3.7 (0.7)		131 (2191)
t-test		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	Mann-Whitney test	$p < 0.001$

*Mean values of serum IgG, IgA (mg/dL), total protein and albumin concentrations (g/dL) with standard deviation (in parentheses) and significance testing in HIV negative and HIV positive subjects. **Median serum levels of IgM (mg/dL) with significance testing (range in parentheses).

Table 2. Comparison of mean Ig levels between HIV positive patients categorized by groups.

Subjects* CD4	n	IgG (mg/dL)	IgA (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
>200	17	1096 (695.7)	176 (81.6)	9.5 (1.5)	3.7 (0.7)
<200	18	926.2(304.7)	165 (56.6)	10.2(1.8)	3.7 (0.8)
t-test		$p = 0.352$	$p = 0.645$	$p = 0.242$	$P = 0.932$

*HIV positive.

Table 3. Median IgM concentration-comparison between groups of HIV positive patients (range in parentheses).

Subjects*	n	IgM (mg/dL)
CD4 > 200	17	131 (483)
CD4 < 200	18	69 (2191)
Mann-Whitney test		$p = 0.177$

*HIV positive.

Though the literature is scanty on the pattern of serum immunoglobulin in HIV-positive, HAART-naïve patients in this environment, the observed mean increase in these immunoglobulins and total protein levels is similar to findings from previous studies in other countries [4-8]. This increase in Ig levels may imply polyclonal B-cell activation with advancing disease [5,8]. Evidence from some laboratories indicate that the viral envelope proteins especially gp41 induce this polyclonal B-cell activation which results in excess abnormal immunoglobulin production [4,5]. The role of this enhanced B-cell response in the presence of HIV infection has been a source of debate for many years. In spite of this observed polyclonal B-cell activation and abundance of immunoglobulins, disease progression to AIDS occurs thereby implying a failure of this humoral response to control the infection [10,12]. This polyclonal activation may reflect direct immune stimulation by one or more components of the virus [10] and the generated antibody pool may consist of neutralizing antibodies and auto-antibodies to a large number of normal cellular proteins [6,13]. Previous studies have indicated that the concentration of most immunoglobulins in HIV-positive subjects of African origin tend to be higher than their counterparts from other parts of the world [10,14]. This variation in immunoglobulin profiles may be genetically determined or may arise from numerous antigenic challenges, especially in the tropics from chronic viral and parasitic antigen exposure. This may result in chronic stimulation of B-cells and increased production of immunoglobulins even in HIV-negative individuals [10,14]. However, the significant drop in serum Ig levels in black African HIV patients on ART compared with the untreated cohort strongly suggest there might be a genetic difference in B-cell response to HIV infection in people of African descent compared with other races [14]. The mean serum IgG level was significantly higher inHIV patients compared with controls. These findings agree with the work of Arinola *et al.* (1998) [6] and are also corroborated by other workers in the African sub-continent. Chronically HIV-infected individuals have been documented to exhibit elevated serum IgG up to two times the normal levels, although the major portion of these antibodies are not HIV specific [13].

Immunoglobulin G is the dominant anti HIV-immunoglobulin isotype and its anti-retroviral subclass reactivity consists mainly of IgG_1 and IgG_3 [13]. One study [8] reported that with the progression to AIDS, the mean serum levels of total IgG, IgG_1 and IgG_2 did not change but the IgG_3 level increased significantly while IgG_4 levels fell significantly. This suggests that monitoring serum total IgG and IgG subclasses may provide crucial information in monitoring disease progression.

The mean serum levels of IgA in this study were found to be 170.4 mg/dL for HIV positive subjects and 106.8 mg/dL for HIV negative subjects. This difference was statistically significant and confirms the findings of pre-

vious studies [4,6,8]. IgA is the most important immu-noglobulin involved in mucosal defenses [4,13]. The same trend on the IgG levels was observed with systemic total IgA levels increasing during progression to AIDS [4]. Polyclonal immune activation and production of specific cytokines (IL 5 and IL 6) appear to be some of the mechanism responsible. However slight elevations of IgA may simply be a reflection of genetic and environ-mental factors within an African population. Common viral infections are initiated by local invasion of epithet-lial surfaces, which initially induces local production of interferon and secretory IgA from these surfaces [10]. Serum IgA antibodies have also been shown to have neu-tralizing activity on HIV [10] but this effect diminishes with disease progression allowing passage of IgA into the blood and resulting in increased serum levels of IgA.

The median serum levels of IgM were also signifi-cantly higher in HIV-positive than HIV negative subjects ($p < 0.001$). This also confirms findings of previous studies [6,8].

In this study, the HIV positive subjects were further stratified into two groups based on their CD4+ T cell count into those with CD4+ cell count < 200 cells/µL and those with ≥200 cells/µL. This was done to compare CD4 cell count with serum immunoglobulin levels among the HIV sero-positive subjects and to determine any as-sociation between CD4 cell counts and observed serum Ig levels. There were no statistically significant differ-ences in these values ($p > 0.05$). These findings are simi-lar to those in a study in Tanzania [7] which reported an insignificant correlation between high serum immu-noglobulin levels and CD4 count.

Low albumin levels have also been associated with HIV disease progression and one study [15] reported that among 453 HIV infected individuals, albumin < 35 g/l (3.5 g/dl) was associated with faster progression to AIDS. Other studies have associated low albumin levels with all-cause mortality in AIDS; however it could not be de-termined whether low albumin levels among these indi-viduals were caused by HIV infection or were reflective of the inherent state of health of the individual. Different hypotheses have been proposed to explain the low levels of albumin seen in HIV infection [15]. Some studies have demonstrated that HIV co-infection with HCV ac-celerates the progression of liver disease while other studies have found albumin to be a significant predictor of HIV disease progression even among those not in-fected with HCV [16]. It is also possible that low that low albumin levels may reflect the effects of anorexia, poor nutritional status or chronic inflammation (albumin is a negative acute phase reactive protein whose levels can possibly be depressed by elevated levels of TNF and IL-1 during chronic inflammation) [15]. In this study, mean serum albumin was 4.3 g/dL among HIV negative

subjects and 3.7 g/dL among HIV positive subjects. This difference was statistically significant. However no sig-nificant difference was observed among HIV positive subjects with CD4 cell count ≥ 200 cells/µL versus those with CD4 counts < 200 cells/µL.

In this study serum IgA, IgG and IgM concentration were non-significantly correlated to viral loads in all HIV positive subjects ($r = –0.206, –0.032$ and $–0.316$ respec-tively).

5. Conclusions

This study confirms that serum levels of total protein, IgG, IgA and IgM are elevated in the course of HIV in-fection while serum albumin levels are lower.

Although the value of immunoglobulin concentration in predicting CD4+ cell count may appear limited on its own, measuring levels in conjunction with other bio-chemical parameters (such as the serum albumin levels) may potentially be used to monitor both disease pro-gression and response to HAART. This might be used to advantage in low resource settings as the cost for anti-body measurements is much lower than that for HIV RNA determination.

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