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Evaluation of immunoglobulin classes (IgA, IgG and IgM) levels and complement fixation activity in HIV infected subjects

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ABSTRACT

The study was designed to evaluate the immunoglobulin A, G and M levels and complement fixation activity in HIV infected participants, who were not administered antiretroviral therapy (ART). Eighty (80) HIV infected participants, aged between 15 – 55 years (38 ±10 years), were recruited for the study. Forty five (45) of the participants were classified as symptomatic HIV (stage ii), while the remaining 35 were classified as asymptomatic HIV (stage i). Similarly, 40 seronegative participants served as control. Blood samples were collected from the participants for the determination of HIV status by immunochromatography, HIV confirmation by Western blot, determination of immunoglobulin levels by immunoturbidimetry, and complement activity by complement fixation test. The IgG and IgA were significantly increased in symptomatic HIV infection compared with asymptomatic HIV infection ($p<0.05$). However, the value of IgM in asymptomatic and symptomatic HIV infected participants were similar ($p>0.05$). The complement fixation activities decreased in HIV infected patients compared with the values in HIV seronegatives. The increase in IgA concentration with HIV infected participants may suggest existence of mucosal infections, while the increase in IgG concentration in HIV infected participants may suggest evidence of increased opportunistic infection requiring IgG response. The low level of complement activity in HIV infected participants may predispose individuals with HIV infection to disease commonly controlled through immunological activity of the complement.

Keywords: Human Immunodeficiency Virus (HIV), immunoglobulins (IgA, IgG and IgM), complements.

INTRODUCTION

Two types of the HIV have been identified namely HIV-1 which is most prevalent causing global epidemic and HIV-2 which is more common in West Africa. The feature of the disease is immune suppression. There have been reports on the effect of HIV infections on several immunological parameters, and the correlation of these parameters with disease progression. Kashala et al. (1993) reported significantly elevated immunoglobulin levels amongst HIV positive individuals compared with HIV negative persons. In view of this report, the present

study assessed the serum immunoglobulin A, G & M levels in HIV infected participants with emphasis on symptomatic (clinical stage ii) and asymptomatic (clinical stage i) states. Also, complement activation had been described in viral lysis, in both HIV infected adults (Perricone et al., 1987) and children (Jarvis et al., 1993). As a result of that, the present study also assessed the complement fixation ability of symptomatic HIV participants (stage ii) and asymptomatic HIV participants (stage i).

MATERIALS AND METHODS

Methods and selection of study population

A total of eighty (80) HIV infected patients were recruited for the study at the Voluntary Counselling and Testing (VCT) and HIV clinic of Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria. They were aged 15-55 years (38 ± 1). Using the WHO classification for HIV infection and CD4 count, 45 of these patients were classified as symptomatic HIV (stage ii), while the remaining 35 were classified as asymptomatic HIV (stage i). Forty (40) apparently healthy HIV seronegative participants drawn from the student and staff population of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria, served as control. Because of the life style it is unlikely that they may have been infected and that the result showed was not that of a window period. Five millilitres of blood was collected from the participants and dispensed into plain tubes for serum for immunoglobulins A, G and M determination by immunoturbidimetry method, using Technicon Ames RA 50 Chemistry Analyzer, and complement fixation assay by complement fixation test. Informed consent was obtained from those who participated in the study. The Nnamdi Azikiwe University Teaching Hospital Board of Ethical Committee approved the study design.

Test for HIV Infection by Immmunochromatography as described by Manufacturers of the kit (Acon Laboratories Inc. USA)

Twenty five microlitres of serum samples was dispensed into the "specimen pad" of the test strip and 80 µl of buffer was added. The reaction was allowed for 5 minutes, the appearance of distinct red lines at test region and control region of the kit suggest positive HIV test while one distinct red line in the region of the control suggest HIV seronegative test. The appearance of the distinct red line of the control region validates the result without which the kit is assumed to be non functional.

HIV confirmatory test by Western Blot

Two millimetres of the reconstituted phosphate buffer pH 8.6 was added to the dish containing the nitrocellulose strip. The cells were incubated for 5 minutes at room temperature with slow shaking. Twenty microlitres of each patient's serum sample was added into the corresponding cell and incubated for 2 hours, at room temperature under slow shaking. The content of each dish was drained and each strip was washed twice for 5 minutes with 2 ml of buffer. 2 ml of enzyme linked anti IgG antibody was dispensed into each cell and incubated for 1 hour and 5 minutes at room temperature with shaking. Two millilitres of colour developing reagent (enzyme substrate) was dispensed into each cell after wasting. The colour reaction was stopped by removing reagent and washed 3 times with buffer as soon as the colour developed. The presence of shades of colour indicates positive results.

Immunoglobulin classes (IgG, IgA, IgM) determination as described by manufacturers of the kit (Human Biochemica and Diagnostica Germany)

Preparation of working calibrator

Aliquot of immunoglobulin of known concentration (IgG, IgA, IgM), as calibrator was diluted with diluent (phosphate buffer pH 7.2) to obtain the following serial doubling dilutions; from 2 to 128.

Preparation of serum samples

Serum samples were diluted 1 in 21 with buffer, pH 7.2 prior to estimation.

Analysis of immunoglobulins (Ig) using Technicon Ames RA 50 Chemistry Analyzer (Germany)

50 µl standard solutions were aspirated into the Technicon, Ames RA 50 chemistry

analyzer and programmed to produce calibrated curve for the standard solutions. Subsequently, each sample prepared was fed into the machine; the machine automatically estimates the concentration of the immunoglobulin in the sample with respect to the standard curve. The values of the immunoglobulin in the samples were digitally displayed.

Complement fixation test

One drop each of patient's serum and their serial doubling dilutions, guinea pigs complement (i.e. Minimum Haemolytic Dose) and the indicator cells (human red blood cell sensitized with anti D antibody) were mixed in precipitin tubes. The tubes were then incubated in the incubator at 37 °C for 2 hours. The titres of complement in the sera were determined by the highest dilution that is still showing haemolysis. Control HIV seronegative serum was processed following the same procedure.

Statistical analysis

The result of the analysis was statistically analyzed. Student's t-test and one-way analysis of variance (ANOVA) were used to compare means. The analyses were performed with the used of SPSS statistical software package. A p value of < 0.05 was considered statistically significant.

RESULTS

Serum immunoglobulin levels

The mean (±SD) serum IgG concentration (mg/dl) was significantly higher in both the symptomatic (10237.1±5796.4) and asymptomatic (6238.6±3711.0) HIV infected participants when compared with the corresponding values in control (1716.5±415.4) ($p<0.05$ in each case). Similarly, the mean (±SD) IgM concentrations were significantly higher in the symptomatic (431.6±244.2) and asymptomatic (316.0±204.2) HIV infected patients compared with the control (162.4±36.2) ($p<0.05$). The mean (±SD) IgA concentration was also significantly higher in the symptomatic (375.3±178.7) and asymptomatic (293.1±96.6) HIV infected patients compared with the control (203.6±78.7) ($p<0.05$ in each case) (Table 1).

The mean (±SD) serum concentration of IgG was significantly higher in symptomatic (10237.1±5796.4) HIV infected

Table 1: Mean (±SD) serum levels of immunoglobulins A, G, M (mg/dl) and complement in HIV infected patients and control participants.

	IgG (mg/dl)	**IgA (mg/dl)**	**IgM (mg/dl)**	**Complement**
Control (n = 40)	1716.5± 415.4	203.6±78.7	162. ±36.2	16±00
Asymptomatic (n =35)	6238.6 ± 3711.0	293.1 ± 96.6	316.0±204.2	6.9±1.8
Symptomatic (n=45)	10237.1±5796.4	375.3±178.7	431.6±244.2	7.3±1.5
f (p)	26.8 (.000)	11.29 (.000)	12.26 (.000)	285.4(.000)
$t1p^a$	p<001	P<0.01	P<0.01	p<0.01
$t1p^b$	p<0.01	P<0.01	P<0.01	p<0.01
$t1p^c$	p<0.01	P<0.05	p>0.05	p>.01

f (p) = symptomatic, asymptomatic and control compared (using ANOVA)

$t1p^a$ = asymptomatic HIV patients compared with control (using t-test)

$t1p^b$= symptomatic HIV patients compared with control (using t-test)

$t1p^c$ = asymptomatic HIV patients compared with symptomatic (using t-test)

participants compared with asymptomatic HIV infected participants (6238.6±3711.0) ($p<0.01$). Similarly, the mean (±SD) IgA concentration in symptomatic HIV infected participants (375.3±178.7) was significantly higher than corresponding value in the asymptomatic HIV infected participants (293.1±96.6) ($p<0.05$) (Table 1).

However, no significant difference was observed in mean serum concentration of IgM between the symptomatic (431.6±244.2) and asymptomatic (316.0±204.2) HIV infected participants ($p>0.05$) (Table 1).

The mean complement activity was significantly lower in both asymptomatic HIV infected participants (7.0±1.8) and symptomatic HIV infected (7.3±1.7) participants compared respectively with corresponding values in the controls (16.0±0.1), ($p<0.05$ in each case). However, there was no significant difference in mean (±SD) complement titre difference between the asymptomatic (7.0±1.8) and symptomatic (7.3±1.7) HIV infected participants ($p>0.05$).

DISCUSSION

Immunoglobulin G, M and A levels

The study observed increased IgG and IgA concentrations amongst HIV infected symptomatic and asymptomatic participants. This was not the case for the serum IgM. The observed increase in serum IgG and IgA suggest the involvement of these classes of immunoglobulin in possible protective immunity. This indicative since there was obvious class switching in favour of IgG and IgA. It may also be an indication for existing opportunistic agents provoking this type of immunological response.

The high increases in serum IgG concentration with disease progression suggest the extent of super antigen interaction with the humoral immune cells and effective immunoglobulin switching. This observed increase in IgG concentration is in agreement with report of Arinla and Igbi (1998) and Orinaasen et al. (2004) who observed that increase in IgG concentration correlated well with disease progression amongst HIV infected patients.

This study also revealed a significant increase in IgA concentration in HIV infected participants. IgA is a secretory immunoglobulin and common viral infection starts with local invasion of epithelial surface, which initially induces the production of secretary IgA from these surfaces. Roitt et al. (2003) and Broliden (2001) reported that resistance to HIV infection in exposed seronegative persons involved HIV–specific mucosal and systemic immune responses. The increase in IgA concentration in HIV infection, however, clearly means that there was increased involvement of IgA in mucosal and submucosal immune defence mechanism. This may likely be the case since the serum IgM concentration was not affected.

The study showed increase in IgM concentration in HIV seropositive participants compared with the HIV negative healthy controls. However, the IgM values remain similar between asymptomatic (clinical stage i) and symptomatic (clinical stage ii) participants. The above pattern of IgM concentration in HIV infection may be attributed to challenge and activation of mature B cells by HIV antigens, which initially produce a relatively non-specific IgM. Thus, it could be that HIV infection present different pattern of antibody response depending on the stage of the disease.

Complement activation had been described in viral lysis, in both HIV – infected adults (Perricone et al., 1987) and children (Jarvis et al., 1993). The complement activities as part of the non-specific humoral immune mediation in HIV infection appear early in HIV infection (Mcknight et al., 1997; Aasa-Chapman et al., 2004). However, Aasa-Chapman et al. (2004) show that IgG antibodies to HIV envelop can lyse viruses through the classical pathway of complement activation. The study has found significant reduced complement levels in both the asymptomatic (clinical stage i) and symptomatic (clinical stage ii) HIV infected patients compared with the HIV seronegative controls as was also reported by earlier workers (Perricone et al., 1987; Jarvis et al., 1993).

Low level of complement found in HIV infection had been attributed to complement consumption by c1q–bound immune complexes (Jarvis et al., 1993), and indeed,

significant elevated levels of circulating immune complexes had been reported in HIV–infected patients in Ibadan (Onyenekwe et al., 2006).

The circulating immune complexes (CIC), bind c1q, causing high activation of complement in the system. The initial high activation of complement may lead to depletion of complement components, which subsequently results in diminished fixation activities in the host. The resultant effect is deposition of circulating immune complexes.

Besides, inducing direct lysis of pathogens, the complement system also has opsonizing, phagocytic – inducing chemo – attractant and immune stimulatory function (Blue et al., 2004). Specific antibodies alone cannot effectively neutralize many viruses, but complement activation can enhance the antiviral effects of antibodies by opsonizing virons or inducing lysis of the particles (Blue et al., 2004).

The decline in complement activity in HIV infection observed in this work suggests that complement mediated immune intervention may be lowered or negligible as they may have been grossly consumed. The finding in this study leads us also to conclude that, complement consumption seem to be an early finding in HIV infection since it was noticed even in asymptomatic HIV patients.

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