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Original Article

Effect of Short Term Antiretroviral Therapy on CD4+ Cells and Immunoglobulins in HIV Seropositive Subjects.

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ABSTRACT

The study was designed to assess the effect of short-term antiretroviral therapy (ART) on IgA, IgG and IgM and CD4 $^{+}$ T cell counts in HIV seropositive subjects. 20 confirmed HIV seropositive subjects, aged between 15-65 years were recruited for the study. They were on triple combinations therapy consisting zidovudine, lamivudine and nevirapine. 20 HIV seronegative subjects were used as control. Blood sample was collected from the participants for the determination of the above parameters. The CD4 $^{+}$ T cell counts show no significant difference between pre-ART and 2 months post-ART ($P>0.05$) but was significantly higher by 4 months post-ART compared with the pre-ART ($p <0.05$). IgG and IgM serum levels showed significantly high values by 2 and 4 months post- ART compared with the pre-ART value ($p <0.05$ in each case). However, the serum IgA level by 4 months post-ART showed no significant difference compared with pre-ART value ($p >0.05$). Meanwhile there were no significant differences in CD4 count, IgM, IgA, and IgG levels between 4 months post-ART values compared with the corresponding values in HIV seronegative control subjects. The present study showed an improvement in the blood concentration of CD4 cell by 4 months post-ART administration, which suggests possible recovery of cellular immunity. The insignificant difference in IgA concentration within the study period possibly suggests non-progressive mucosal or sub mucosal infections. Similarly the raised IgM and IgG concentrations within the study period may be an indication of existing infections and signifies possible potentials towards short-term recovery. This shows that with the use of these drugs prognosis seem good for the short term.

Keywords: ART, RVD, human.

INTRODUCTION

HIV infection is wide spread in the sub Saharan Africa1[1,2]. The viral infection has been shown to affect both the number and functions of the CD4+ T cells, thus causing impaired T cell functions[3,4]. At the same time hypergammaglobulineamia have be1en noticed in patients with human immunodeficiency virus (HIV) infection. They have been shown to exhibit a generalized non–HIV specific polyclonal B–cell activation with increased production of HIV specific antibodies[5,6,7,8,9]. However, the use of ART has shown good promise because it effectively suppresses the HIV in vivo replication, restoring CD4+T cell number and functions. Consequently the use of ART has reduced both HIV morbidity and mortality rates. Presently, there is coordinated use of combined ART in Nigeria. The present study was thus designed to understand the recovery of CD4+ T cell and total immunoglobulin A,G,M concentration before and during commencement of the combined ART.

MATERIALS AND METHODS

Subjects:

20 confirmed HIV seropositive subjects (male = 5 and female = 15), aged between 15 – 65 years were recruited for the study and followed up bimonthly for 4 months. 5mls of blood was collected from the participants at each visit and dispensed into EDTA tubes for CD4+ T cell count and into plain tube for serum extraction for immunoglobulin (G, M, A) determinations. 20 HIV seronegative subjects were used as control. The participants gave informed consent and the study design was approved by Nnamdi Azikiwe University Teaching Hospital (NAUTH) Board of Ethical Committee.

METHODS

HIV Screening by Immunochromatographic Method: The procedure was as described by the manufacturers of the kit (CHEMBIO diagnostic system, Inc. New York USA). In brief, 5μl of serum samples was dropped into the "specimen pad" of the test strip. Then 80μl of buffer was then added. The reaction was allowed for 10 minutes, the appearance of distinct red line at the 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 seronegativity. The appearance of the distinct red line at the control region validates the result without which the kit is assumed to have deteriorated.

Cyflow Counter Automated CD4+ Count: 20μl of EDTA whole blood was collected into Partec test tube (Rohren tube). Then 20μl of CD4+T antibody was added into the tube .The contents were mixed and incubated in the dark for 15 minutes at room temperature. 800μl of CD4 buffer was gently added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the CD4+T cells were displayed as peaks and interpreted as concentrations.

Immunoturbidimetric Method of Immunoglobulin - A,G, M, Estimations

Protocol for Determination of IgA:

The procedure was as described by the manufacturer of the kit (Human, Germany). 900ul of the phosphate buffer (PH 7.2) was delivered in a test tube and 3ul of sample added into the tube. The same procedure was performed for the standard IgA. The reaction was mixed and incubated at 37^0c for 5 minutes in water bath. It was read as A1 spectophotometrically. Subsequently, 80ul of anti-IgA reagent was added into the same tube, mixed and incubated at 37^0C in water bath for 5 minutes. It was then read spectophotometrically and recorded as A2. The result was read off the standard immunoglobulin graph plotted with concentration against the absorbance using various dilutions of the standard immunoglobulin.

Method for Determination of IgG by Spectrophotometry

The procedure was as described by the manufacturers of the kit (Human, Germany). 400ul of the phosphate buffer PH 7.2 was delivered in a test tube and 4ul of sample was added to the tube. The same procedure was performed for the standard IgG serum. The reactions was mixed and incubated at 37^0c for 20 minutes in water bath. It was read as A1 spectrophotometrically. Subsequently, 15ul of anti IgG reagent was added into the same tube, mixed and incubated at 37^0c in water bath for 20 minutes. It was then read and recorded as A2. The result was read off the standard immunoglobulin graph plotted with concentration against the absorbance using various dilutions of the standard immunoglobulin.

Method for Determination of IgM by Spectrophotometry.

The procedure was as described by the manufacturer of the kit (Human, Germany). 900ul of phosphate buffer PH 7.2 was delivered

in a test tube and 4ul of the sample was added to the tube. The same procedure was performed for the standard IgM serum. The reaction was mixed and incubated at 37^0c for 20 minutes in water bath. It was read as A1 spectrophotometrically. Subsequently, 150ul of anti IgM reagent was added into the same tube, mixed and incubated at 37^0c in water bath for 20 minutes. It was then read spectrophotometrically and recorded as A2. The result was read off the standard immunoglobulin graph plotted with concentration against the absorbance of the standard dilutions of the immunoglobulin.

RESULTS

There was no significant difference in mean CD4+ T cell counts (/mm^3) in HIV positive subjects between pre–ART value of 348±216 and 2 months ART value of 408±227) ($p>0.05$). similarly, no significant difference in mean value of serum IgA was seen between the pre-ART era (240.2±82.1) and 2 months post–ART era (220.7±46.9) $p>0.05$. This was also the case for both IgG and IgM during the same period of evaluation. See table 1.

By 4 months post ART era, no significant difference in mean value of CD4+ T cells (484±225) was observed compared with value during pre-ART era ($p>0.05$). there was also no significant difference in mean serum IgA concentration by 4 months post ART era (212.5±56.0) compared with pre-ART value ($p>0.05$). However, the mean serum IgG concentrations (mg/dl) by 4 months post–ART era (784.4±183.1) was significantly higher compared with the pre-ART value (568.1±339.0) ($p<0.05$). Similarly, the serum IgM concentration (mg/dl) by 4 months post–ART (151.9±49.7) was significantly higher than the corresponding value pre-ART (108.0±35.0) ($p<0.05$). See table 2.

The mean CD4+T cell count (/mm^3), and mean serum concentrations of IgA, IgG and IgM by 4 months post ART era were similar to their corresponding values in control HIV seronegative subjects ($P>0.05$ in each case). See table 3.

Table 1: The mean blood CD4+ T cell count (/mm^3), serum concentrations (mg/dl) of IgA, IgG and IgM in HIV seropositive subjects pre–ART and 2 months post– ART

Parameter	HIV Patients pre – ART (n = 20)	HIV Patients 2 months post – ART (n = 20)	P – value
CD4 count (per ul)	348.2±216	408.0±227	>0.05
IgA (mg/dl)	240.2±82.1	220.7±46.9	>0.05
IgG (mg/dl)	568.1±339.0	732.1±220.1	>0.05
IgM (mg/dl)	108.0±35.0	119.4±32.0	>0.05

Table 2: The mean blood CD4+ T cell count (/mm^3), serum concentrations (mg/dl) of IgA, IgG and IgM in HIV seropositive subjects pre–ART and 4 months post– ART

Parameter	HIV Patients pre – ART (n = 20)	HIV Patients 4 months post – ART (n = 20)	P – value
CD4 count (per ul)	348.2±216	494.2±225	<0.05
IgA (mg/dl)	240.2±82.1	212.5±56.0	>0.05
IgG (mg/dl)	568.1±339.0	784.4±183.1	<0.05
IgM (mg/dl)	108.0±35.0	151.9±49.7	<0.05

Table 3: The mean blood CD4+ T cell count (/mm^3), serum concentrations (mg/dl) of IgA, IgG and IgM in HIV seronegative subjects and HIV seropositive subjects at 4 months post- ART.

Parameter	HIV negative control subjects (n = 20)	HIV Positive Patients 4 months post – ART (n = 20)	P – value
CD4+ T cell (per ul)	537.4±116	494.2±225	>0.05
IgA (mg/dl)	183.7±52.8	212.5±56.0	>0.05
IgG (mg/dl)	699.7±343.0	784.4±183.1	>0.05
IgM (mg/dl)	125.4±34.5	151.9±49.7	>0.05

DISCUSSION

The present study observed an improvement in the blood concentration of CD4 +T cell by 4month post-ART administration. This suggests possible recovery of cellular immunity within short term ART combination therapy. CD4 + T cells are cells known to control both the cellular and humoral immune responses. However, these immune cells are the principal target of the invading HIV.

The invasion of these immune cells by the HIV causes CD4 dysfunction and death. This will subsequently result in reduction of CD4 + T cell population. In the present study, the administration of ART combination for short term led to the recovery of the CD4 + T cell population this might be an indication of possible impact of the ART at limiting the viral replication processes in the infected hosts. Similar finding has been reported independently by Resino et al.[10] and neckolic et al.[11].

The serum IgA concentration showed no significant different within the study period. The non significant different in IgA concentration observed within the study period possibly suggest, non-progressive mucosal or submucosal infection. The observed similar concentration between the Pre-ART treatment and 4-month post-ART treatment may also suggest non-deterioration in mucosal and sub-mucosal immunity and integrity upon commencement of ART. The non-progressive or drop in mucosal infection may mean an index of good prognosis in HIV infected individuals on ART.

However, this study observed raised IgM and IgG concentration within the study period. This might be possibly due to the opportunistic infections, usually associated with HIV infection. The existing systemic infection may have activated B cell proliferation and differentiation into plasma cells, and shedding of IgM immunoglobulin against the antigens. Similarly, the raised IgG shows that there was effective immunoglobulin class switching leading to the production IgG that is mainly involve in secondary immune response. The high production of the immunoglobulin M and G may signify possible potential towards short term ART administration. Studies elsewhere have shown that HIV causes hyper activation of B cells[5,6,12]. The finding of the present study suggest that the hyper gammaglobulineamia encountered in HIV subjects on ART are mainly IgG and IgM induced but not IgA induced.

The present study thus concludes that there is possible recovery of cellular immunity and progressive recovery of humoral immunity in HIV subjects following short term ART combination administration.

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