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Original article

Evaluation of CD4$^+$T CELL COUNT, Interleukin 6 (IL-6) and interferon gamma in HIV infected symptomatic and asymptomatic individuals.

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ABSTRACT

The study was designed to assess the effect of HIV infection on CD4$^+$T cell count, IL-6 and IFN-γ in HIV infected individuals. Forty-five HIV positive subjects (male 27, female 18), aged between 15-65 years were recruited for the study. 21 of this (male 10, female=11) were HIV symptomatic individuals, while the remaining 24 subjects (male=17, female=7), were HIV asymptomatic individuals; 22 HIV seronegative subjects served as control individuals. Blood samples collected from all the individuals that participated in the study were used for the determination of HIV status by immunochromatographic method, CD4$^+$T cell count by Flow Cytometry, IFN-γ and IL-6 by ELISA method. The result showed diminished CD4$^+$T cells count and serum concentration of IFN-γ in HIV symptomatic individuals ($P<0.05$ in each case). Meanwhile, the serum IL-6 concentration was similar amongst the three groups ($p>0.1$). The drop in both the CD4$^+$T cell count and serum concentration of IFN-γ in the HIV symptomatic individuals suggests possible progression in impairment of certain immunologic responses during HIV infection.

Keywords: cytokines; HIV; CD4

INTRODUCTION

HIV infection is known to alter expression of cellular protein via modulation of cytokine expression, which in turn could alter immune responses to viral infection. It is also known that cytokines play a crucial role in the pathogenesis of HIV infection[1]. Cytokines have complex effects on the replication of HIV, and conversely in infected individuals, also HIV directly affects cytokine production. Different report suggested that the progression of disease in HIV infected individual may be controlled by the balance between the levels of type 1 (Th1) and type2 (Th2) cytokine[2,3]. Some workers had suggested that progressive defects in interleukin 2 (IL-2), IL-12 and IFN-gamma production and the increased production of IL-4 (IL-4-driven helper IgE), IL-6 and IL-10 (Th2) could provide another correlate of disease progression[2,3,4]. Often a shift from a strong Th1/weak Th2 to a weak Th1/strong Th2 profile has been observed[4]. However, there is one report on the lack of evidence of switching of cytokine profile from Thito Th2[5]. But Prasad et al[6] reported increased levels of interleukin 6 (IL-6), interferon gamma and IL-2 RNAs and decreases macrophage inflammatory protein lB (MLP 1B) in infected thymocytes. Cytokine production from T lineage cells is also altered by HIV infection[6].

Anti CD3/anti-CD28-activated T - lymphocyte from HIV-1- infected individual showed a decrease in interferon gamma and IL-13 secretions compared with cells from uninfected individuals. Expression of these cytokines was increased when patients presents with bacterial or viral infections[7]. This study was designed to evaluate the CD4$^+$T cell count; serum concentrations of IL-6 and IFN-γ in HIV asymptomatic and symptomatic individuals.

STUDY APPROACH AND METHODOLOGY

Subjects: A total of forty-five HIV seropositive individuals were recruited for the study at the HIV Clinic, Nnamdi Azikiwe University Teaching Hospital Nnewi. 21 of these individuals were grouped according to WHO criteria for staging HIV into: HIV symptomatic (stage 2) group (n=21) and HIV asymptomatie (stage 1)

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group (n=24). Similarly, 22 apparently healthy HIV seronegative individuals served as control group for the study. The individuals that participated in the study gave informed consent and the NAUTH ethical committee approved the study design.

Sample collection: 4mls of Blood sample collected from each participant was dispensed respectively into EDTA tubes for CD4$^+$T cell count and into plain tubes for HIV screening, and determinations of serum IL-6 and IFN-γ concentrations.

METHODS

HIV Screening: Two different methods were used; Abbott determine^{Tm} 1&2 which is an in-vitro visually read immunoas say and immunochromato graphic test for the qualitative detection of antibodies to HIV- 1 and HIV-2 in human plasma. For the Abbott determine^{Tm} HIV- 1 & 2 the procedure as described by the manufacturer was used for the analysis. Briefly, 50iil of participants' plasma samples separated from corresponding whole blood samples in EDTA were applied to appropriately labeled sample pad. After 15 minutes of sample application, the result was read. This method has inherent quality control that validates the results. Two visible red colours in the region labeled control and patient represents HIV sero-positive reaction while a single red colour in the region labeled control represents HIV sero-negative reaction. For the immunochromato graphic method for HIV 1 & 2 it utilizes immobilized antigen for the detection of antibodies to HIV - 1 & 2 in the plasma. It is used as a point of care test and suitable for use in multi-test algorithms. The procedure as described by the manufacturer was used for the analysis. In brief, a 5ml plasma sample loop provided was used to collect the participants' plasma by touching it on the specimen and allowing the opening of the loop to fill with the liquid. The samples were dispensed into the sample wells in appropriately labeled sample pad. Three drops of the buffer supplied by the manufacturer was added drop-wise into the appropriately labeled sample wells. The results of the tests were read at 10mins after the addition of the running buffer. This method has inherent quality control that validates the results. The presence of two pink/purple lines in the region of test sample and control indicates HIV seropositive reaction while a single pink/purple line at the control region indicates HIV seronegative reaction. HIV sero-positive results' using these two methods was used to classify participants as presenting with HIV infection.

CD4$^+$T cell Count by Flow Cytometry Machine: 20ml of blood sample in EDTA tube was collected into Partec test tube. Then 20ml of CD4$^+$T antibody was added into the tube .The contents were mixed and incubated in the dark for 15 minutes at room temperature. 800ml 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 the count determined.

Interferon gamma assay: The procedure was as described by the manufacturers of the interferon gamma assay kit[8]. 100µl of serum sample was added to wells of microtiter plate and 50µl of biotinylated detection antibody was also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated for 2 hours at room temperature and 100µl of streptavidin conjugate added to various wells, and were further Incubated for 30 minutes at room temperature. 100ml of ready to use TMB was added and the microtiter plate, incubated in the dark for 15 minutes for the colour to develop. 100µl H_2SO_4 was added in the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank.

Calculation:

Absorbance of test/absorbance of standard x concentration of standard = concentration of test.

HUMAN IL-6 BY ELISA

RESULTS

The mean IFN-γ of 40.8±8.3 in control group and 45.5±12.6 in HIV asymptomatic group, showed no significant difference ($p>0.01$). Similarly, the mean IL-6 of 15.1 ± 3.2 in control group and 15.4 ± 3.4 in HIV asymptomatic group were not significantly different ($p>0.1$). See table 1.

The mean IFN-γ of 25.5 ± 11.1 in HIV symptomatic group was significantly reduced compared to 48.8±8.3 observed in the control group ($p<0.05$). However the mean IL-6 value in HIV symptomatic and control group were not significantly different ($p>0.05$). See table 2.

The mean IFN-γ was significantly reduced in HIV symptomatic group compared with the HIV asymptomatic group 45.5±12.6 ($p<0.01$). However, there was no significant difference in IL-6 between the HIV symptomatic and HIV asymptomatic groups ($p>0.1$). Similarly the mean $CD4^+$ T cell counts was significantly reduced in HIV symptomatic group compared with HIV asymptomatic groups ($p<0.05$).

Table 1: Comparison of mean (±SD) Interleukin - 6, and Interferon--γ in HIV asymptomatic and control groups.

Parameters	Control group (n=22)	HIV asymptomatic group (n=24)	P -value
Interferon-γ	40.8 ±8.3	45.5 ±12.6	>0.1
Interleukin - 6	15.1 ±3.2	15.4 ± 3.4	>0.1

Table 2: comparisons of mean (±SD) Interleukin -6, and Interferon-γ in HIV symptomatic and control groups.

Parameters	Control (n=22)	Symptomatic HIV positive (n21)	P- value
Interferon-γ	40.8± 8.3	25.5±11.1	<0.01
Interleukin-6	15.1± 3.2	14.2± 5.6	>0.1

Table 3: comparison of mean (±SD) Interleukin-6, Interferon-γ and $CD4^+$T cell count in HIV asymptomatic and HIV symptomatic groups.

Parameters	HIV asymptomatic (n=24)	HIV Symptomatic (n=21)	P- value
Interferon-γ	45.5± 12.6	25.5± 11.1	<0.01
Interleukin -6	15.4 ±3.4	14.2±5.6	>0.1
$CD4^+$T cell count	397±164	162± 99	<0.01

DISCUSSION

The present study demonstrated decreased Interferon-g production in HIV symptomatic individuals. Thi cells usually secrete the interferon-γ when they are stimulated by specific antigen complex presented by macrophages. The possible decline in production of IFN- γ could be due to impaired antigen presentation or due to reduction in CD4 T cell count and function usually seen in progressive HIV infection.

Decrease in IFN-γ in symptomatic HIV subjects have been reported in several studies of HIV infected subjects[2,3,9,10]. The implication of these reports is defective cellular immunity. This may result in effective viral replication. Similarly, impaired nitroblue ingestion by neutrophils has been reported in HIV infected subjects suggesting possible defective antigen presentation. Hence defective secretion of IFN-γ seems to commence as early as stage 2 HIV infection but not observable in asymptomatic HIV individuals.

However, the serum concentration of IL-6 in both the HIV symptomatic and HIV asymptomatic individuals were similar to that observed in the apparently health individuals. This possibly an indication that HIV infection may not affect production of IL-6. In this study IL-6 was used as index for TH-2 cells secretions and possible functions. The finding of serum levels of IL-6 in both the HIV symptomatic and HIV asymptomatic individuals may suggest that at this stage of HIV progression antibody production by infected host might have not been compromised. IL-6 which is usually secreted by Th2 cells is known to be involved in B cells stimulation for antibody production against the HIV specific antigens and other opportunistic infections.

However, studies elsewhere did show increase in IL-6 concentration in HIV infected subjects. Thus confirming that antibody secreting ability of HIV infected subjects at this stage is still effective[3,10]. However, in another study, no significant change was observed in serum concentration of IL-6 in HIV infected

individuals as disease progresses The discrepancy in the findings for IL-6 may be an indication of progressive changes in immunological dynamics depending on possible pattern of secondary infections in the respective studied groups.

In the present study, mean $CD4^+$ T cell count was reduced in HIV symptomatic individuals and was just about half of the value recorded for the HIV asymptomatic individuals. The magnitude of drop in $CD4^+$ T cell count in HIV symptomatic individuals may be an indication of degree of collapse of the immune response particularly the TH-1 type as the disease progression is recorded. This massive drop in $CD4^+$ T cell count may also possibly reflect drop in CD 4 $^+$T cell functions. This may possibly explain the drop in IFN-γ as observed in the HIV symptomatic individuals.

Since cell mediated immunity has been shown to play an important role in controlling viral infection [12] such collapse may encourage viral replication in infected host. Using IFN-γ and IL-6 as markers of TH-1 and TH-2 immune responses in HIV infected individuals, this study suggest possible down-regulation of TH-1 pathway of immune mechanism which are necessary for effective immunity against HIV infection. However, it is important to the limitations of using single markers as index where there are multiple networks of cytokines that controls TH-1 or TH-2 immune mechanisms.

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