CD4:CD8 T-cell Ratio and Full Blood Count Status of Humanimmunodeficiency Virus (HIV) and Pulmonary Tuberculosis (TB) Patients in Three Centers with Voluntary Testing and Counseling Units in Delta State, Nigeria.

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ABSTRACT

Two hundred and five patients suspected of Humanimmunodeficiency virus (HIV) infection and or Pulmonary Tuberculosis (TB) and 100 apparently healthy subjects were recruited into the study from February 2006 to February 2008 to determine the relevance of CD4:CD8 T-cell ratio and full blood counts as diagnostic indices of the immunological status of HIV and TB subjects in parts of Delta State, Nigeria. HIV status was determined using (WHO) System two and Ziehl Neelsen staining techniques were used for tuberculosis screening. Automatic Cyflow counter was employed for the estimation of CD4:CD8 ratio and standard haematological technique was used for full blood count measurements. The mean CD4 of 220.7 19.5, 445.1 33.0 and 205.2 30.5 for HIV, TB and HIV/TB respectively were significantly different ($p < 0.05$) when compared with 880.4 + 49.3 for controls. Likewise the mean CD8 + of 724.8 + 106.0 for HIV and mean T-cell of 0.4 + 0.0 for HIV/TB infected subjects were significantly different $P < 0.05$) when compared with 401.6 22.3 and 2.8 for controls respectively. The mean PCV of 25.9 0.6, 27.5 0.5 and 25.0 0.9, mean neutrophil of 62.9 1.6, 62.6 1.4, 63.7 2.8; mean lyumphocyte of 36.0 1.5, 34.7 1.3 and 34.3 7.0 for HIV, TB and HIV/TB were also significantly different ($P < 0.5$), when compared with 35.9 0.3, 54.9 0.9 and 44.5 0.9 for controls respectively. The mean eosinophi count of 6.7 1.7 for tuberculosis was significantly higher ($P < 0.05$) when compared with 2.02 0.2 for controls. This study further confirms CD4:CD8 + ratio and lymphocytes count as good diagnostic indices for HIV and TB infections in Delta State, Nigeria.

Keywords: CD4+:CD8+ ratio, Human immunodeficiency Virus, Tuberculosis.

INTRODUCTION

In acute Human immunodeficiency Virus (HIV)-infection, the virus replicates extensively in the absence of any detectable adaptive immune response, reaching levels of over 100 million copies of HIV RNA/ml. It is during this cycle of viral replication that important pathogenic processes are thought to occur (1). The number of CD4 + T cells decline, occasionally to levels that allow the development of opportunistic infections (2,3). In addition to the decline in CD4 +T cell counts, qualitative impairments of CD4 +Tcell function are perhaps the most characteristic abnormalities detected in HIV infection and occurs very early in acute infection (4-6), potentially due to the preferential infection of virus-specific CD4 + T cells by the virus (7). This subsequently results in a functional impairment of HIV-specific CD8+ T cell (6).

A massive oligoclonal expansion of CD8 + cell response has been described during acute HIV infection (8), and the appearance of HIV specific CD8 + T cells has been temporally associated with the initial decline of viremia (9,10). These CD8 +T cells have the ability to eliminate HIV-infected cells directly by MHC class I-restricted cytolysis or indirectly by producing cytokines, chemokines or soluble factors, thus curtailing the generation of new viral progeny (11).

While most opportunistic infections occur in the advanced stages of HIV infection, patients can develop tuberculosis at any stage regardless of the levels of circulating CD4 + T-cells (12). Over 50% of cases with tuberculosis occur in patients with CD4 + counts of more than 200 cells/ul in the peripheral blood (13).

An individual's vulnerability to any disease depends on the strength of the immune system, which is affected by nutrition, stress and presence of other infections and parasites as well as other factors. Malnutrition undermines immune response, directly increasing vulnerability to disease (14).

However, cytopaenia is a common complication of infection with HIV and in the course of the disease more than 70% of the patients develop anemia, frequently requiring transfusion (15). Neutropaenia and lymphopaenia are regularly seen indicating that more than one haematopoietic lineage may be impaired, and the degree of cytopaenia often reflects severity of the disease (16). Most people with HIV have leucocyte counts at low normal end of the range. On the average, a healthy adult has between 4,000- 11,000mm-3(17).

In view of the above, the present work was initiated to assess the immune status of HIV and Tuberculosis patients as a diagnostic index in Delta State, Nigeria.

MATERIALS AND METHODS

This study was conducted in three foci of Delta State, with Voluntary Testing and Counseling facilities namely; Central Hospital Kwale, Central Hospital Agbor,

Tuberculosis and Leprosy Referral Centre Eku. The study areas of Agbor, Eku amd Kwale lie approximately between longitude 5o00' and 6o45'East and latitude 5o00' and 6o30' North of Delta State. The population of the study area are: Agbor: 109,204; Eku:113,929 and Kwale: 114,117(18).

Sampling

Five milliliters of venous blood and sputum samples were collected into 0.1ml of EDTA and clean universal containers respectively from 205 patients suspected of HIV infection or Tuberculosis and 100 apparently healthy subjects by stratified random sampling method. Whole blood was used for full blood count, and plasma for the determination of the subjects' HIV sero-status. Informed consent was obtained from each subject while approval for the study was obtained from ethical committee of Delta State Ministry of Health.

Sample Analysis

HIV screening test was carried out using two enzyme linked immunosorbent assay rapid screening kits based on WHO systems two for detecting antibodies to HIV-1 and 2 (18). Determine Rapid Screening kits (Abbott Laboratories, Japan) and Immunocomb II (Organics, France) were used in the study. Tests were carried out according to manufacturer instructions. Sputum samples were examined for *Mycobacterium tuberculosis* using Zeihl Neelsen staining method (19). Automatic method using CyFlow Counter(Partec GmbH, Germany) was used for the determination of CD4+ and CD8+ cell counts (20) on all subjects. Haematocrit method and Leishman staining technique were carried out on all blood samples collected for the estimation of packed cell volume, total and differential leucocyte counts respectively (19). All the data generated were subjected to statistical analysis using the one way analysis of variance (ANOVA) and Dunnett's multiple comparison tests (Post test).

RESULTS

Our results showed that the mean CD4+ counts of infected subjects; 220.7± 19.5 for HIV; 455.1±33.0 for TB; and 205.2± 30.5 for HIV/TB were significantly lower ($P<0.05$), when compared with 880.4± 49.3 for controls respectively. The mean CD8+ of 724.8± 106.0 for HIV and the mean T-cell of 0.04±0.0 for HIV/TB were significantly different ($P<0.05$), when compared with 401.6±22.3 and 2.8±0.2 for controls respectively (Table 1).

The mean CD4+ counts of infected subjects; 202±22.7 for HIV, 348.1±46.3 for TB and 154.5±31.0 for HIV/TB in Kwale were significantly lower ($P<0.05$), compared with 880.4±49.3 for controls. Also the mean CD4+ counts of 240.0±37.15 for HIV; 250.1±49.3 for TB and 321.4±65.7 for HIV/TB subjects in Agbor were significantly different ($P<0.05$), compared with 880.4± 49.3 for controls. Similarly, the mean CD4+ counts of 212.1±37.8 for HIV; 560.1±47.1 for TB and 239.7±61.9 for HIV/TB in Eku were significantly lower ($P<0.05$), when compared with 880.4±49.3 for controls (Table2).

Table 2 showed that the mean CD4+ for HIV, TB and HIV/TB in Kwale, Agbor and Eku were reduced respectively while the mean CD8+ were elevated in the same duration when compared with each other and controls. There is a far less disproportionate response of CD4+ and CD8+ and T-cells to TB infections in the study.

The mean PCV, neutrophil and lymphocytes for HIV,TB and HIV/TB were significantly different ($P<0.05$) when compared with controls (Table 3).

TABLE 1: Mean S.E.M CD4+, CD8+ and T-cells ratio of HIV, TB and HIV/TB co-infected patients and control subjects in the study

Parameters (no./µl)	Controls (n=100)	HIV positive (n=75)	TB positive (n=67)	HIV and TB positive (n=34)	F-cal	P-value
CD4 cells	880.4±49.3	220.7±19.5*	455.1± 33.0*	205.2±30.5*	72.6	P<0.05
CD8 cells	401.6±22.3	724.8±106.0*	421.7± 25.3	561.1±56.8	5.4	P<0.05
T-cells	2.8±2.0	1.3±0.8	1.7±0.35	0.4±0.0*	2.5	P<0.05

P<0>0.05; *=P<0.05 for variables in the same row (Using Dunnett's Multiple Comparison Test)

TABLE 2: Mean ± S.E.M CD4+, CD8+ and T-cells ratio of HIV, TB and HIV/TB infected patients in each center

Centre	No tested	Parameters (no/µl)	Controls (n=41)	HIV positive (n=35)	TB positive (n=27)	HIV and TB (n=13)	F-cal	P-value
Kwale	78	CD4	880.4 ± 49.3	202.8 ± 22.7*	348.1 ± 46.3*	154.5 ± 31.0*	41.38	P<0.05
		CD8	401.6 ± 22.3	788.4 ± 276.1*	417.9 ± 41.0	506.1 ± 67.7	2.06	P<0.05
		T-cell	2.8 ± 2.0	0.4 ± 0.0*	1.1 ± 0.2*	0.3 ± 0.0*	31.06	P<0.05
			(n=32)	(n=20)	(n=32)	(n=8)		
Agbor	60	CD4	880.4 ± 49.3	240.0 ± 37.15*	250.1 ± 49.3*	231.4 ± 65.7*	32.70	P<0.05
		CD8	401.6 ± 22.3	757.4 ± 69.6*	587.5 ± 94.8	702.6 ± 134.7*	13.18	P<0.05
		T-cell	2.8 ± 2.0	2.3 ± 1.7	0.6 ± 0.1	0.4 ± 0.1	0.8	P<0.05
			(n=27)	(n=20)	(n=18)	(n=13)		
Eku	67	CD4	880.4 ± 49.3	212.1 ± 37.8*	560.1 ± 47.1*	239.7 ± 61.9*	32.32	P<0.05
		CD8	401.6 ± 22.3	539.5 ± 66.7	385.3 ± 30.47	527.1 ± 103.6	3.03	P<0.05
		T-cell	2.8 ± 2.0	0.7 ± 0.3*	2.2 ± 0.6	0.5 ± 0.2*	4.7	P<0.05

P<0>0.05; *=P<0.05 for variables in the same row (using Dunnett's Multiple Comparison Test)

TABLE 3: Mean ± S.E.M heamatological indices of HIV, TB and HIV/TB infected patients and control subjects

Parameters	Controls (n=100)	HIV positive (n=75)	TB positive (n=67)	HIV and TB positive (n=34)	F-cal	P-value
PCV	35.9 ± 0.3	25.9 ± 0.6*	27.5 ± 0.5*	25.9 ± 0.9*	87.0	P<0.05
WBC	4648.0 ± 127.0	4164.0 ± 234.1	5480.9 ± 475.9	5080.4 ± 518.7	3.2	P<0.05
NEUT.	54.9 ± 0.9	62.9 ± 1.6*	62.5 ± 1.4*	63.7 ± 2.8*	7.7	P<0.05
LYMPH.	44.5 ± 0.9	36.0 ± 1.5*	34.7 ± 1.3*	34.4 ± 2.7*	11.8	P<0.05
EOSIN.	2.0 ± 0.2	3.9 ± 0.4	6.4 ± 1.7*	3.3 ± 0.7	3.5	P<0.05

P<0>0.05; *=P<0.05 for variables in the same row (using Dunnett's Multiple Comparison Test)

DISCUSSION

Our study showed that the mean CD4+ counts were reduced in HIV, TB and HIV/TB infections, while the mean CD8+ were elevated. This agrees with earlier findings (2,3) that CD4+ T cells decline in HIV infection and pave way for the development of opportunistic infections. This low level of CD4+ cells may be attributed to the qualitative impairment of the CD4+ cell functions (4-6) and also the preferential infection of virus-specific CD4+ T cells by HIV (7). Subsequently, there is an increase in CD8+ cells of HIV specific CD8+ T cells (6) as a result of functional impairment which is temporarily associated with the initial decline of viremia (9,10). This however, may be due to the ability of the CD8+ cells to eliminate the HIV infected cells indirectly by MHC class 1-restricted cytolysis or directly by producing cytokines, chemokines or soluble factors thus curtailing the generation of new viral progeny (11).

The differences in the mean CD4+ CD8+ obtained in Kwale, Agbor and Eku may be attributed to nutrition, stress and presence of other infections. This is in consonance with the earlier findings (14) that malnutrition undermines immune response and directly increases vulnerability to disease. The mean CD4+ of 455.1±33.0 obtained for TB is higher than the mean CD4+ of 220.7±19.5 and 205.2±30.5 recorded for HIV and HIV/TB respectively. This is also in consonance with the findings (13) that over 50% of tuberculosis cases are patients with

CD4 counts of more than 200 cells /μL of peripheral blood. This is because in HIV/TB infection, the CD4+ cells are direct target of the virus, in addition to direct seeding of the heamatopietic cells (5) which leads to rapid decrease in the CD4+ T cells. Although, there was far less disproportionate response of CD4+, CD8+ and T-cells in TB infections at the centers, patients can develop TB at any stage, regardless of the levels of circulating CD+ T cells as earlier reported (12).

The low levels of PCV, neutrophil and lymphocyte observed in this study agree with the findings (17,18) that cytopaenia is a common complication of HIV and TB and that in the course of the disease more than 70% of the patients develop anaemia, frequently requiring transfusion. This phenomenon may also be an indication that more than one haematopoietic lineage may have been impaired and the degree of cytopaenia often reflects severity of the disease (15). The mean leucocyte counts observed in the study were significant and within normal range. This agrees with the report (16) that most people with HIV,HIV/TB have leucocyte counts at the low normal end of the range.

It therefore becomes imperative that for good care and management of HIV and tuberculosis patients, the assessment of the immune status via estimation of CD4+ , CD8+ and full blood counts is necessary. Our results showed significantly raised levels ($P<0.05$) of eosinophils in TB infections. Hence there is need for more work to be done in this area to ascertain the functional and numerical significance of eosinophils in tuberculosis. In addition, the possibility of producing a vaccine or immunotherapeutic agents based on the roles of CD4+ and CD8+ cells in pathogenesis of HIV infection remains to be explored as a major open avenue for disease control and management.

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