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Full Length Research Paper

Evaluation of CD4+ and CD8+ T-lymphocyte counts and serum levels of immunoglobulins in pulmonary tuberculosis (PTB) patients with or without HIV co-infection in South Eastern Nigeria

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The prevalence and incidence of tuberculosis (TB) has risen throughout sub-Saharan Africa with increasing human immuno-deficiency virus (HIV) prevalence. TB patients were recruited from some treatment centres in South Eastern Nigeria, and a total number of 133 subjects were recruited for the study. The following parameters, namely, CD4+ and CD8+ T-cell counts, immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) serum levels were determined using the cytoflow method, Dynal T8 quantitative method as well as single radial immunodiffusion (SRI), respectively. The results showed that the mean CD4+ T-cell count (mm^3) (338.34 ± 36.65) and CD8+ T-cell count (mm^3) (278.70 ± 27.80) of pulmonary tuberculosis (PTB) patients with HIV co-infection (PTB + HIV) patients were significantly lower than the mean, CD4+ T-cell count (mm^3) (788.18 ± 58.17) and CD8+ T-cell count (mm^3) (421.17 ± 30.68) of control subjects. The mean CD4+ T-cell count (mm^3) (614.14 ± 58.24) and CD8+ T-cell count (mm^3) (388.43 ± 32.07) of PTB patients were significantly lower than those of the control subjects. The mean CD4+ T-cell count (mm^3) (388.77 ± 63.62) and CD8+ T-cell count (mm^3) (564.18 ± 93.68) of HIV seropositive patients were significantly lower than CD4+ T-cell count of the control subjects, but higher than CD8+ T-cell count of the control subjects ($P < 0.05$). The mean IgA (mg/dl) level of in PTB patients (234.96 ± 48.00) was significantly higher than those of PTB patients with HIV seropositive (PTB + HIV) (164.01 ± 42.27). The mean IgG (mg/dl) level of PTB + HIV patients (1446.37 ± 277.32) was significantly higher than that of PTB patients (1092.18 ± 114.25). The mean IgM (mg/dl) level in HIV seropositive patients (212.08 ± 22.84) was significantly higher than that of PTB patients (156.69 ± 32.07), PTB + HIV (182.76 ± 46.77) patients and control subjects (93.58 ± 21.18) ($P < 0.05$). It is therefore evident that PTB infection with or without HIV co-infection significantly affects the immune system response. The immunological changes observed showed that PTB infection might have cause the moderate decrease in CD4+ and CD8+ T-lymphocyte counts, while the decrease is well marked in PTB infection with HIV co-infection.

Key words: Pulmonary tuberculosis (PTB), human immunodeficiency virus (HIV), immunologic changes, immunoglobulins.

INTRODUCTION

Tuberculosis (TB) is an old disease of man and one of the most widespread and persistent disease in this century (1900 to 2000) (WHO, 2005). Some reports over the past several years have indicated that, while the TB problem is on the wane in most developed countries, the disease is still rank a major health problem in the developing countries of the world in general and in sub-Saharan Africa in particular (WHO, 2005; Abdul and Andrew, 2009). Nigeria is a country with high incidence of tuberculosis with or without human immuno-deficiency virus (HIV) co-infection, and pulmonary tuberculosis (PTB) problem in Nigeria is still enormous (Jason et al., 2000; Jemikalajah and Okogun, 2009). Earlier surveys in some parts of the country have confirmed a high prevalence of the disease by tuberculin testing. This study was therefore designed to evaluate some immunologic markers, such as CD4+ T-cell count, CD8+ T-cell count, serum immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) levels in PTB patients with or without HIV co-infection.

MATERIALS AND METHODS

A total number of 133 subjects were recruited for this study: PTB + HIV = 33 (males = 18, female = 15); PTB = 30 (males = 15, female = 15); HIV seropositive = 28 (males = 14, females = 14) and control subjects = 42 (males = 22, females = 20). The CD4 and CD8 lymphocyte counts were carried out as well as the serum IgA, IgG and IgM levels. The PTB patients (63) were recruited from treatment centres in Mile 4 Hospital, Abakaliki, Ebonyi State (41); Our Lady of Lourdes Hospital, Ihiala (6); Iyienu Specialist Hospital, Ogidi (6) and Nnamdi Azikiwe University Teaching Hospital, Nnewi (10). The control subjects (42) were selected from staff and students of Nnamdi Azikiwe University, Nnamdi Azikiwe University Teaching Hospital and staff of Mile 4 Hospital. The participants gave their informed consent.

Laboratory procedures

Ethylenediaminetetraacetic acid (EDTA) blood (3 ml) and 7 ml whole blood were collected from confirmed patients with PTB, using a 10 ml syringe and 21 g needle, all sterile. This was after the puncture area was identified, swabbed with alcohol and then was collected by well trained phlebotomists. The EDTA blood sample collected was processed within 24 h, at room temperature, for CD4+ and CD8+ T-cell counts. The CD4 count was done by cytoflow method, whereby the CD4+ cells were stained with fluorochrone, and when they become energized, through the laser beam, the CD4 cells were scattered, and with the help of the photomultiplier tube (PMT) they were displayed as numbers or as histograms, and the result was read. When 50 µl of EDTA blood was dispensed into a Partec test tube, 10 µl of CD4 antibody was added, mixed and incubated in the dark for 10 to 15 min. After incubation, 800 µl of diluted buffer was added and vortexed. The mixture was then passed via the suction pipe and the result was obtained as histograms on computer read out system.

The CD8+ T-cell count was performed by Dynal T8 quantitative method of SL Green by Partec Germany. The Dynabeads were coated with monoclonal antibodies, used to isolate CD8+ lymphocytes directly from EDTA anticoagulated whole blood, and a pre-depletion of monocytes with Dynabeads CD4 ensures removal of monocytes expressing the CD8 antigens, leaving only the T-lymphocytes expressing CD8 antigen for better accuracy. Haemocytometer was finally used to count the CD8+ T-cells which were calculated using the dilution factor. The serum immunoglobins (IgA, IgG and IgM) were performed using single radial immunodiffusion (SRI) test. The agarose gel plate contains the antigen for the specific proteins to be analyzed. After air-drying the plates, 5 µl of the serum of test samples were placed into the appropriate wells and were labeled accordingly. At the end of 72 h, the IgA and IgG precipitin ring diameters were read to the nearest 0.1 mm. The IgM plates were read at the end of 96 h, according to the recommendation of the manufacturer, Linear Chemicals. The CD4+ T-lymphocytes (338.34 ± 36.65) and CD8+ T-lymphocytes (278.70 ± 80) of patients with PTB + HIV were significantly lower than those of the control subjects ($P < 0.05$). The CD4+ T-lymphocytes (614.14 ± 58.24) and CD8+ T-lymphocytes (388. 43 + 32.07) of patients with (PTB-HIV) were significantly lower than those of the control subjects ($P<0.05$). The CD4+ T-lymphocytes (388.77 ± 63.62) of HIV seropositive patients were significantly lower than that of the control subjects ($P < 0.05$).

Statistical methods

Data obtained were analyzed using student t-test and analysis of variance (ANOVA). The variables were expressed in mean standard deviation (±SD) and the significant difference was regarded as $P < 0.05$.

RESULTS

CD4 and CD8 lymphocyte count

Table 1 summarizes the changes in the levels of CD4+ T-cells and CD8+ T-cells in patients with PTB with or without HIV co-infection when compared with those of the control subjects. The mean CD4 count of patients with PTB+HIV (338.34 ± 36) and that for HIV seropositive patients (388.77 ± 63) were significantly lower than those of control subjects (788.18 ± 52). The CD4+ T-cells of PTB patients (614.14 ± 58) was also significantly lower than that of control subjects ($P < 0.05$). Hence, PTB with or without HIV co-infection significantly affects the immune system response.

IgA, IgG and IgM levels changes

Table 2 summarizes the changes observed in the level of serum IgA, IgG and IgM. The mean IgA serum level of patients with PTB (284.96 ± 48) and patients with PTB + HIV (164.01 ± 42) were significantly higher than

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Table 1. CD4 and CD8 T-lymphocytes changes in PTB with or without HIV co-infection.

Category	n	CD4 (μl^{-1})	CD8 (μl^{-1})
PTB+HIV	33	338.34 ± 36.65[b]	278.70 ± 27.80[b]
PTB-HIV	30	614.14 ± 58.24[c]	388.43 ± 32.07
HIV seropositive	28	388.77 ± 63.62[b]	564.18 ± 93.68
Control	41	788.18 ± 52.17[a]	421.17 ± 30.68
F	-	8.296	2.981
P	-	0.000	0.006

[a]P < 0.05 significantly higher than in PTB+HIV. [b]P < 0.05 significantly lower than in PTB-HIV. [c]P<0.05 significantly higher than in HIV seropositive.

Table 2. Immunoglobulin changes in PTB with or without HIV co-infection.

Category	n	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
PTB-HIV	30	284.96 ± 48.18	1092.18 ± 14.25	156.69 ± 32.67
PTB+HIV	33	164.01 ± 4227	1446.37 ± 277.32	182.76 ±47.77
HIV seropositive	28	110.71 ± 25.15	1361.67 ± 151.62	212.08 ± 22.84
Control subjects	42	67.51 ± 14.24[a,b]	560.96 ± 73.21[a,b,c]	93.58 ± 21.18[a,b,c]
F	-	5.157	4.437	3.325
P	-	0.000	0.001	0.009

[a]P < 0.05 significantly lower than PTB-HIV patients. [b]P < 0.05 significantly lower than PTB+HIV patients. [c]P<0.05 significantly lower than in HIV seropositive patients.

those of HIV seropositive patients (110.71 ± 25) and control subjects (67.51 ± 14) (P < 0.05). The mean IgG level in patients with PTB+HIV (1446.37 ± 277) and HIV seropositive patients (1362.6 ± 151) were significantly higher than those of the control subjects (560.96 ± 73) (P < 0.05). The mean IgM level of HIV seropositive patients (212.08 ± 22) was significantly higher than that of control subjects (93.58 ± 21) (P < 0.05). One could therefore monitor the treatment of patients with PTB-HIV with IgA serum level, PTB+HIV with IgG serum level and HIV seropositive patients with IgM serum level. Table 3 summarizes the changes in CD4+ T-cell and CD8+ T-cell levels in PTB patients with or without HIV co-infection with gender analysis.

The mean CD8+ T-lymphocyte count of female patients with HIV seropositive (744.71 ± 168.71) was significantly higher than that for male patients with HIV seropositive (388.64 ± 93.68) (p < 0.05). The mean CD4+ T-lymphocyte count of male (330 ± 36.65) and female (346.67 ± 89.94) patients with PTB+HIV were significantly lower than those of male (634.35 ± 62.59) and female (593.92 ± 58.24) patients with PTB-HIV (P < 0.05) as well as male (779.44 ± 58.93) and female (780.91 ± 52.17) of the control subjects (P < 0.05).

DISCUSSION

It was observed in this study that there was a significant decrease in CD4+ and CD8+ T-lymphocyte counts in male and female PTB patients with or without HIV co-infection. This is in agreement with previous reports by some authors who reported a general depression of CD4+T cell in the peripheral blood of PTB patients (Onwubualili et al., 1987; Tripathy et al., 2000). Tripathy et al. (2000) revealed that the CD4+ and CD8+ T-lymphocyte counts were significantly decreased in PTB patients, as well as in patients with acute malaria and other infections, and this may be due to cytokine level imbalance or the presence of acute phase proteins. Another reason for the decrease might be due to interferon gamma secreting CD4+ and CD8+ T-cells recruited to the lungs during PTB infection (Feng et al., 1999; Serbina and Flynn, 1999). An investigator also reported a CD4+ T-lymphocytopenia in severe pulmonary TB without evidence of HIV infection (Pilheu et al., 1997). A significant increase in CD8+ T-lymphocyte count of female patients with HIV seropositive when compared with that of their male counterparts observed in this study is in agreement with previous report by Prims et al. (1999) who suggested a relationship between the immune response system and sex hormonal influences.

A significant increase in IgA level in PTB patients was observed in this study. This must be due to the primary role of IgA in mucosal immunity (Abdul and Andrew, 2009). This is in conformity with that reported by Mayne (1994) which stated other immunoglobulins diseases of the gastro intestine tract and respiratory tract,

Table 3. CD4 and CD8 lymphocyte changes in PTB patients with or without HIV co-infection with gender analysis.

Category	Sex	n	CD4 (μl^{-1})	CD8 (μl^{-1})
PTB+HIV	Male	18	330 ± 36.65[b]	226.57 ±27.80[b]
	Female	15	346.67 ± 89.94[b]	330.83 ±132.71
PTB-HIV	Male	15	634.35 ± 62.59[c]	377.83 ± 32.07
	Female	15	593.92 ± 58.24[c]	399.02 ± 24,57
HIV seropositive	Male	14	376.36 ± 71.68[b]	383.64 ± 168.71
	Female	14	401.18 ± 63.62[b]	744.71 ± 168.71[a]
Control	Male	21	779.91 ± 58.93[a,c]	437.78 ± 39.26
	Female	20	780.91 ± 52.17[a,c]	404.55 ± 30.68
F	-	-	8.296	2.810
P	-	-	0.000	0.009

[a]$P < 0.05$ significantly higher than in PTB+HIV. [b]$P < 0.05$ significantly lower than in PTB-HIV. [c]$P<0.05$ significantly higher than in HIV seropositive.

especially tuberculosis and bronchietasis. Andrea et al. (2008) also reported that IgA was observed to be the most abundant antibody class and provides the first line of defence in mucosal secretions. Growing evidence suggested that IgA uses a high affinity binding system to neutralize microbial toxins and pathogens and a low affinity binding system to prevent commercial bacteria from breaching the mucosal surface (Macpherson et al., 2008; Singh et al., 2009).

The mean IgG level of PTB patients with and without HIV co-infection was significantly higher than that of control subjects in this study. This must be due to the prime function of IgG in opsonization, complement activation and antibody dependent cell-mediated cytotoxicity (Abdul and Andrew, 2009). This finding is in agreement with that of Swati et al. (2006), who observed that children suffering from tuberculosis of the central nervous system, showed 85% sensitivity of IgG antibody and 90% for TB antigen in cerebrospinal fluid. Some workers observed that IgG level increased in HIV infection, this must also be due to the prime function of this antibody class in opsonization, complement activation and antibody dependant cell mediated cytotoxicity.

In this study also, there was no significant increase in IgM level observed in PTB patients with or without HIV co-infection. This must be due to the primary role of IgM in naïve B cell antigen receptor and complement activation (Abdul and Andrew, 2009), IgM level was significantly increased in HIV seropositive patients. This is in conformity with the report by Reithman and Franked (1982) which suggested a marked decrease in IgM in PTB disease. It was also reported that IgM is the first immunoglobulin produced by the lymphocytes before the switch to IgG. IgM is frequently associated with the immune response to antigenically complex blood-born infectious organisms (Abdul and Andrew, 2009); hence, the significant increase in HIV seropositive patients.

Conclusion

This study showed that immunologic markers: CD4, CD8, IgG, IgA and IgM are significantly affected in PTB with and without HIV co-infection, and could be used as monitoring tools.

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