*Full Length Research Paper*

# The co-infection of human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* among patients seen at the general hospital Umuguma, Owerri, Imo State, Nigeria

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In this study, the co-infection of Human Immunodeficiency Virus (HIV) and *Mycobacterium tuberculosis* was investigated among patients. HIV serology was performed on 100 tuberculosis patients whose sputum samples were AFB positive as tested at the General Hospital Umuguma, Owerri, Imo State, between 2nd May 2010 and 3rd February 2011. Seven (7.0%) of the 100 tuberculosis patients were HIV seropositive. HIV seropositivity was higher in females than in males (ratio, 5:2) and the groups infected were between 18 to 42 years. The HIV seropositive tuberculosis patients had signifcantly lower median total leucocyte count (2,700/mm$^3$) compared with that of the healthy control group (6,000/mm$^3$). Also the median percentage lymphocyte count was significantly lower in those seropositive (18%) when compared with the healthy control group (38%). The study shows that *M. tuberculosis* remains a high risk factor in HIV/AIDS infection in Nigeria. Routine HIV screening test of all tuberculosis patients is highly recommended.

**Key words:** *Mycobacterium tuberculosis* infection, HIV, risk factor, Nigeria.

## INTRODUCTION

Zimmer (2000) observed that man is a collection of cells that work together and kept harmonized by chemical signals. If a microorganism can control these signals, then it can control man: these views are well documented in Zimmer's DO PARASITES RULE THE WORLD? Some parasites such as pneumocystis, toxoplasmosis and cryptosporidium are among the most common opportunistic infections in patients with AIDS (Roberts and Janovy, 2000). Infection with Human Immunodeficiency Virus (HIV) causes a progressive and ultimately profound reduction in the cell mediated immune response, thus setting the stage for a variety of opportunistic infections that are normally held in check by the defence mechanism (Koenig and Fanci, 1988).

*Mycobacterium tuberculosis* is one of these pathogens and the diseases caused by it have become a common presenting infection in patients with Acquired Immunodeficiency Syndrome (AIDS). The symptoms of tuberculosis in HIV seropositive patients are similar to those encountered in HIV seronegative tuberculosis patients. Fever and night sweats are the most common complaints and other associated symptoms such as weight loss, anorexia and chills are frequently reported. A chronic wasting syndrome without any localizing signs or symptoms may be the only clue to tuberculosis in any patient with or without HIV infection.

HIV is a lymphocytopathic retrovirus that preferrentially infects T-Lymphocytes that express the CD4$^+$ antigen (Kiatzmann et al., 1984). In the early stages of HIV infection, the numbers of circulating CD4+ cells are normal. Subsequently, however, there is progressive reduction in their number and in the final stage, AIDS patients typically present with opportunistic infections that

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correlate with a severe depletion of CD4 lymphocytes (David et al., 1988). An elevation in the peripheral CD8^{+} lymphocyte count is typical of infection with HIV and results in an inversion of CD4^{+}: CD8^{+} ratio.

Coinfection of HIV and some micro-organisms have been studied and reported by many researchers. Trichomoniasis has been studied and *Trichomonas vaginalis* implicated as a risk factor for HIV/AIDS (Acholonu et al., 2006). Esumeh et al. (2009) studied the prevalence rates of Hepatitis B virus and HIV infections among blood donor in Benin City, Nigeria and reported that Hepatitis virus is a risk factor to HIV infection.

The association of *M. tuberculosis* with HIV in immunodeficiency situations was studied by Edeki and Nwobu (1996) in Idi-Araba, Lagos State. It was reported that tuberculosis was the first clinical manifestation of cellular immunodeficiency in 88% of HIV seropositive patients. The relative virulence of *M. tuberculosis* probably results in its appearance as an opportunistic pathogen earlier in the course of HIV induced immuno-suppresions than other organisms such as *Pneumocystis carinii* (Charles et al., 1990). HIV serological testing should be considered for patients found to have a positive tuberculin test. Conversely, tuberculin skin testing should be performed on HIV infected patients when they are diagnosed as seropositive, particularly in patients from population with an increased risk of *M. tuberculosis* infection (Edeki and Nwobu, 1996).

We aim to investigate the frequency of occurrence of HIV and *M. tuberculosis* among patients in Owerri where information is very scanty.

## MATERIALS AND METHODS

### Subjects

A total of 100 patients (60 males and 40 females) within the ages 6 to 65 years with newly diagnosed cases of tuberculosis (acid and alcohol-fast bacilli, AAFB) were enlisted for this study. Patients were screened for AFB between 2nd May 2010 and 3rd February, 2011. Each patient was interviewed and answers were recorded through questionnaires so as to obtain reliable demographic information, behavioural risk data and medical history. A total of 100 healthy individuals (60 males and 40 females) were used as controls and matched with the patients examined.

### Specimen collection

Sputum and blood samples were both collected from patients and controls. A total of 100 (5 ml) freshly voided sputum samples were produced through deep cough and collected in sterile plastic containers. Equally, 100 (8 ml) blood samples were collected and analyzed for HIV, total leucocyte and differential leucocyte counts. Blood sample (4 ml) collected were quickly introduced into plain vacutainer tubes and the another 4 ml mixed with potassium ethylene diamine tetracetic acid ($K_2$ + EDTA) for total leucocytes count and differential leucocyte count while serum was obtained after centrifugation of the vacutainer tube at 3,500 rpm for 1½ min at room temperature for HIV test.

### HIV antibody screening

All human sera were screened for HIV antibodies by the enzyme-linked immunosorben-assay (ELIZA) using the HIV 1 & 2 Bispot (Orgenics Israel) samples showing positive reactions were further confirmed with immunocomb HIV 1 & 2 test method. Acid and alcohol fast staining test was done (Ziehl-Nelson Reaction) using the acid-fast property of mycobacteria to detect them in sputum. Specifically, heat-fixed smears of sputum samples are flooded with solution of carbol fuchsim (mixture of basic fuchsim and phenol) and heated until steam rises. After washing with water, the slide is flooded with 3% HCl and further processed as stated by David et al. (2002). Red bacilli are seen against the contrasting background colour. Total leucocyte count is performed as previously described by Lewis and Dacie (1984).

Leashman staining method was done according to the method of David et al. (2002).

## RESULTS

The study involved the screening of 100 acid and alcohol fast bacilli cases which were sex and age-matched with controls. Of the 100 tuberculosis patients, 7(7%) of the patients had positive antibodies to HIV while 93(93%) had no antibodies to HIV (Table 1). The risk of HIV infection was significantly greater for females than for males, since 5 females and 2 males were implicated to be having HIV-associated with tuberculosis. The patients implicated in these cases were between 18 and 42 years (Table 1). Of the positive cases identified, 3 of the 5 HIV-associated TB female patients had received transfusion of blood and blood products in 2010 while the other 4 HIV associated TB patients (2 females and 2 males) could have been exposed to any HIV transmission risk factor such as congenital transmission, indiscriminate sex, barbing hazards etc. These established the presence of HIV transmission risk factors. Results from stained positive sputum smears showed no difference in HIV seropositive TB patients and HIV seronegative TB patients. This is because acid and alcohol fast bacilli (AAFB) were seen in sputum smears of all 100 patients.

The mean total leucocyte count (TLC) established from 100 control individuals was 6,000 WBC/mm³ (6,000 ± 3,000 WBC/mm³) compared to the mean TLC in the HIV seronegative TB patients of 2,700 WBC/mm³. The median neutrophil count in the HIV seropositive TB patients was 77% (77±8) and that of HIV seronegative TB patients revealed marked neutrophilia compared to normal control neutrophil count of 60% (mean). The median lymphocyte count of HIV sero-positive TB patients was 19% (19 ± 0: Range 13 to 25%) compared to normal control lymphocyte mean value of 38% (38 ± 13: Range 25 to 51%) and the median lymphocyte count of HIV sero-negative TB patients was 37% (37 ± 14: Range 23 to 51%) (Table 1).

## DISCUSSION

In the early stages of HIV infection, the numbers of

**Table 1.** Parameters tested in patients with HIV and tuberculosis co-infection (MEAN ± SD).

| Parameter | Controls | Tuberculosis (TB) patients | HIV seropositive TB patients | HIV seronegative TB patients |
|---|---|---|---|---|
| N (%) | 100 | 100 | 7(7.0) | 93(93.0) |
| AAFB (3x) | | Positive | Positive | Positive |
| HIV (3x) | | Positive (7) negative(93) | Positive | Negative |
| Age (Years) | 34 ± 15.2 | 33.4 ± 15.0 | 30 ± 12 | 33.4 ± 16 |
| Range | 19 - 49.4 | 18 − 48.4 | 18 − 42 | 17 − 49 |
| Total Leucocyte Count/mm$^3$ | 6,000 ± 3,000 | 5,300 ± 3,000 | 2,700 ± 1,000 | 4,800 ± 2,500 |
| Range | 3,000 − 9,000 | 2,300 − 8,300 | 1,700 − 3,700 | 2,300 − 7,300 |
| **Differential leucocyte count (%)** | | | | |
| Neutrophils | 60 ± 10 | 69 ± 13 | 77 ± 8 | 75 ± 10 |
| Range | 50 − 70 | 56 − 82 | 69 − 85 | 65 − 85 |
| Lymphocytes | 38 ± 13 | 29 ± 18 | 19 ± 6 | 37 ± 14 |
| Range | 25 − 51 | 11 − 47 | 13 − 25 | 23 − 51 |
| Monocytes | 0 ± 2 | 0 ± 2 | 0 ± 2 | 0 ± 2 |
| Range | 0 − 2 | 0 − 2 | 0 − 2 | 0 − 2 |
| Eosinophils | - | - | - | - |
| Range | 0 − 1 | 0 − 1 | 0 − 1 | 0 − 1 |
| Basophils | 0 | 0 | 0 | 0 |

AAFB: Acid and alcohol fast bacilli; HIV: human immunodeficiency virus; SD: standard deviation; N: number of samples.

circulating CD4+ cells are normal. Over the time, however, there is progressive reduction in their number and in the end stage AIDS patients typically present with opportunistic infectious that correlate with a severe depletion of CD4 lymphocytes (David et al., 1988). The present study showed that 7(7.0%) of 100 Nigerian tuberculosis patients examined had detectable antibodies to Human Immunodeficiency Virus (HIV). This contrasts with results of a similar study that established significant HIV seropositive prevalence of 17(28%) out of only 60 tuberculosis patients screened at the San Francisco Department of Public Health Tuberculosis Clinic. Subsequently, many researchers have reported relatively low figures for Nigerian tuberculosis patients. Okafor (1982) screened over 230 adult Nigerian's blood samples and reported a HIV prevalence of 1.5% among the tuberculosis patients. A similar study at Tebellong Hospital, Lesotho in 1998 reported a HIV seropositivity of 52% among the patients with tuberculosis. Further, the HIV seropositive figure 7(7.0%) of 100 tuberculosis patients, however, exposes M. tuberculosis pathogen as a high risk factor for infection with HIV and AIDS among Nigerians.

In this study HIV seropositivity was more frequent in females than in males. This is significantly variable with the report recorded by Afrano et al. (2007), which reported higher prevalence in males than females. The HIV seropositivity was observed in patients between the ages of 18 and 42 years (Table 2) and this correlates with the findings of Theuer (1990) and Hernandez-Pando et al. (2007) who had earlier postulated that the immune response plays a

**Table 2.** Age, sex and leucocyte count distribution of HIV seropositive tuberculosis patients.

| No | Age distribution of HIV associated tuberculosis patients (years) | Sex | WBC (mm$^3$) | Differential leucocyte count (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | N | L | M | E |
| 1 | 46 | M | 2,200 | 77 | 22 | 1 | |
| 2 | 18 | M | 2,000 | 79 | 20 | - | 1 |
| 3 | 26 | F | 2,400 | 80 | 19 | - | 1 |
| 4 | 19 | F | 2,700 | 78 | 20 | - | 2 |
| 5 | 40 | F | 4,400 | 81 | 19 | - | |
| 6 | 29 | F | 2,100 | 64 | 36 | - | |
| 7 | 38 | F | 2,300 | 75 | 25 | - | |

Mean age: = 30; SD of population = 12; Range = 30 ± 12; = 18 – 42 years. WBC/mm$^3$ = White blood count; N% = neutrophil percentage; L% = lymphocyte percentage; M% = monocyte percentage; E%= eosinophil percentage.

fundamental role in the outcome of *M. tuberculosis* infection.

There was moderate neutrophilia in both HIV seropositive tuberculosis patients and HIV seronegative tuberculosis patients (Table 2) when compared with the control subjects.

The total leucocyte count of the HIV seropositive tuberculosis patients was also significantly reduced when compared with that of the health control group (Table 2). However, there was no significant difference between the total leucocyte count of the HIV seronegative tuberculosis group. This reveals the fact that the cellular immune system of HIV seronegative tuberculosis patients are to a large extent not destroyed by the infecting mycobacteria organisms, especially in the early stages of *M. tuberculosis* infection, rather it only persists and replicates within non activated host macrophases (Montgomery and Leman, 1933).

It is recommended that all patients with tuberculosis in Nigeria should undergo HIV antibody testing to ensure that all cases of tuberculosis-related HIV infections are promptly identified and given appropriate medical and public health attention.

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