

Full length Research Paper

Prevalence of *Mycobacterium tuberculosis* and human immunodeficiency virus (HIV) infections in Umuahia, Abia state, Nigeria

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The prevalence of *Mycobacterium tuberculosis* (TB) and human immunodeficiency virus (HIV) infections was investigated among individuals from age 16 years and above attending out patient clinic in Federal medical centre (FMC) and other hospitals in Umuahia. A total of two hundred and fifty individuals were examined. The examinations of the samples from the patients were done according to bacteriological and hematological standards. The overall prevalence of *M. tuberculosis* and human immunodeficiency virus (HIV) infections was 21.6 and 14.0% respectively. The prevalence of patients with TB/HIV co-infections was 6.4%. Males have a higher TB/HIV prevalent rate (3.6%) than females (2.8%). There was no significant difference ($P = 0.01$) in prevalence of *M. tuberculosis* and human immunodeficiency virus infections among the sex group. There was also no significant difference between age groups for *M. tuberculosis* infections but there was a significant association between age and human immunodeficiency virus infections. *M. tuberculosis* and HIV infections were significantly high among the individuals with anemia ($P = 0.01$). All the individuals infected with *M. tuberculosis* and HIV had significantly elevated erythrocyte sedimentation rate (ESR > 51 mm/hr) ($P = 0.01$). None of the individuals with *M. tuberculosis* and HIV infections had normal erythrocyte sedimentation rate (ESR 3 - 8 mm/hr). The highest rate of infection for both *M. tuberculosis* and HIV was among the age group 26 - 35 years. *M. tuberculosis* significantly caused more anemia (PCV, 20 - 25%) compared to HIV infection. HIV and *M. tuberculosis* infections are of public health importance and need effective control especially among the young age group.

Key word: *Mycobacterium tuberculosis*, human immunodeficiency virus.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* and occasionally *Mycobacterium bovis*. The bacterium causes lesions called tubercles and are often called tubercle bacilli. It primarily attacks the lungs in more than 80% of the cases, leading to primary tuberculosis. Extra-pulmonary tuberculosis occurs in less than 20% of cases and affects various organs such as lymph nodes, the meninges, intestine, bone and joints among others (Obionu, 2007). From the World Health Organization global TB programme

report, 30 million people will die in the next 10 years (WHO, 2007). *M. tuberculosis* infection remains the leading infectious killer of youth and adults.

The human immunodeficiency virus (HIV) is the etiologic agent of acquired immune deficiency syndrome (AIDS). HIV is a lentivirus within the family Retroviridae (Brooks et al., 2002). The first case of HIV/AIDS in Nigeria was reported in 1986. Since then the number of people living with HIV or AIDS (PLWAs) steadily increased with an increase of seroprevalence from 1.8% in 1991 to 5.8% in 2001 (Federal Ministry of Health, 2005). Providing geographical spread, Lambo (2004), reported that HIV incidence in North - Central Zone was 0.2%, North - West 0.3%, North - East 0.4%, South -

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East 0.2%, South – South 0.2% and South - West 0.3%. The average national HIV prevalence rates in Nigeria, based on sentinel surveillance study, uses antenatal clinic attendees as proxy to the general population. In 2001, HIV prevalence among the 15 -19 year old antenatal clinic attendees tested was 5.9% among the 20 - 24 years olds the rate was 6.0% and among the 25 - 29 year old, 6.3% (Federal Ministry of Health, 2001).

A close and complex association exists between tuberculosis (TB) and HIV infections. Tuberculosis is the leading cause of death in people with HIV and also has an adverse effect on HIV progression (Obionu, 2007). People who are infected with both TB and HIV are 25 - 30 times more likely to develop TB disease than people infected only with TB (WHO, 2007). *M. tuberculosis* infection in people with HIV can progress to active TB disease very quickly. People with HIV are at risk of being infected if they are exposed to *M. tuberculosis* because their weakened immune system makes them more vulnerable (AIDS Action, 1995). HIV increases susceptibility to infection with *M. tuberculosis*. Sentinel surveillance in Nigeria in 2001 showed the prevalence of HIV among TB patients to be 19.1%. The HIV seroprevalence in TB patients worldwide has underscored the urgent need to screen all suspected TB patients for HIV infection. Therefore the objectives of this study were to determine the prevalence of TB, HIV and TB/HIV coinfection among individuals from 16 years and above. Also to determine the packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) of the blood samples from the individuals.

MATERIALS AND METHODS

Population of study

The population group covered during this study was men and women aged 16 and above, who attended the general out-patient department (GOPD) in FMC Umuahia and other peripheral clinics in Umuahia, Abia State. The total sample size was 250 individuals. It is pertinent to mention that those aged 16 - 49 years are the productive work force of any nation while women in this age bracket are the reproductive group and are therefore a good proxy for the general population in the determination of the prevalence of TB, HIV and TB/HIV coinfection.

Detection of HIV in blood samples

In order to detect HIV in blood samples, human immunodeficiency virus rapid test kits (ACON[®], U.S.A.) was employed. The kit is a rapid chromatographic immune assay qualitative for the detection of antibodies to HIV type 1 and type 2 in whole blood, serum or plasma. Immuno comb II HIV 1 and 2 combfirm kit (ORGENICS, Israel) was also used for confirmation of an initial reactive human serum or plasma specimen. Blood specimen was collected from each patient using sterile needle and syringe and introduced into a clean specimen container. The containers were labeled appropriately with name, sex and age of patients as well as time

and date of collection. The blood specimen, the test strip, buffer and controls were allowed to equilibrate at room temperature (15 - 30°C) prior to testing. The test strip was removed from the foil pouch and the tape on the test card peeled off and the test strip stock in the middle of the test card. The dropper was held vertically and one-drop of venipuncture whole blood was transferred to the "specimen pad" of the test strip and then two drops of buffer were applied. The initial time was recorded and then the result read after 15 min. The result was regarded as positive if two distinct coloured lines appeared on the strip. A positive result was confirmed by using the Immuno comb II HIV 1 and 2 combfirm kit (Orgenics, Israel), an indirect solid - phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each card has 6 pairs of teeth, with six antigen spots per pair (3 spots on each tooth). The left tooth of each pair carries an upper spot sensitized with human immunoglobulin (internal control) and the two protein markers p24 (gag) and p31 (pol). The right tooth has three protein spots gp36, gp 41 and gp 120. The developing plate has 6 rows (A-F), each roll containing a reagent solution ready for use at a different step in the assay.

The test was performed stepwise, by moving the card from row to row, with incubation at each step. To start the test, serum specimens were added to the diluents in the wells of row A of the developing plate. The card was then inserted in the wells of row A. Anti-HIV antibodies, if present in the specimens will specifically bind to the HIV antigens on the teeth of the card. Unbound components were washed away in row B. In row C, the anti-HIV IgG captured on the upper spots (internal control), will react with anti-human IgG antibodies labeled with alkaline phosphatase (AP). In the next two rows (D and E) unbound components were removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components.

The results were visible as gray-blue spots on the surface of the teeth of the card. Interpretation of the results was thus: the appearance of only the internal control indicated that the corresponding specimen was negative for antibodies to HIV-1 or HIV- 2. Specimens which yielded a minimum of two circular, coloured HIV antigen spots on the strip indicated HIV- positive.

Ziehl Neelsen (ZN) stain for *Mycobacterium tuberculosis*

The sputum specimens were collected in sterile wide mouthed screw capped containers. The sputum specimen smear was fixed on the slide. The fixed smear was then flooded with strong carbon fuchsin stain for 3 min and then heated without boiling, rinsed with tap water and decolourised with acid-alcohol for 3 - 5 s, washed again with water and counter- stained with Löffler's methylene blue. It was rinsed again with tap water, allowed to dry and then examined microscopically using oil immersion lens (x100). Bacilli appeared as red - beaded rods 2 - 4 mm long and 0.2 - 0.5 mm wide in positive sputum smear cases (Brooks et al., 2002). Where the number of tubercle bacilli were few or undetected by Z - N stain, the culture method of diagnosis using Lowenstein - Jensen medium (solid egg medium) was used in the isolation of *M. tuberculosis*.

Culture of sputum samples

Culture method is the standard for the diagnosis of *M. tuberculosis* (Wilson and Miles, 2003). In this study, Lowenstein - Jensen medium (solid egg medium) was used. The specimen (sputum) homogenized and decontaminated by modified Petroff's method, was inoculated onto Lowenstein - Jensen medium, incubated at 37°C for upwards of eight weeks. Smears were made from each culture plate with growth and stained for AFB using Z-N stain.

Table 1. Age and sex distribution of patients.

Age (year)	Male (%)	Female (%)	Total (%)
16 - 25	37 (14.8)	30 (26.0)	67 (26.8)
26 - 35	35 (14.0)	39 (15.6)	74 (29.6)
36 - 45	31 (12.4)	27 (10.8)	58 (23.2)
46 - 55	13 (5.2)	16 (6.4)	29 (11.6)
> 56	11 (4.4)	11 (4.4)	22 (8.8)
Total	127 (50.8)	123 (49.2)	250 (100)

RESULTS

A total number of 250 persons attending outpatient clinic at Federal medical center (FMC) and other clinics in Umuahia were examined (Table 1). Out of the total number 127 (50.8%) were males while 123 (49.2%) were females. There were more patients in the 26 to 35 years age group, 74 (29.6%) while age group 46 years and above have less number of patients 51 (20.4%). Table 2 showed that the overall prevalence of *M. tuberculosis* was 54 (21.6%) of which 31 (12.4%) were males and 23 (9.2%) were females. There was no significant different at $P = 0.01$ between sex and *M. tuberculosis*. Table 2 also showed an overall prevalence of 35 (14.0%) for HIV of which 18 (7.2%) were males and 17 (6.8%) females. There is also no significant association between sex and HIV infection. The prevalence of patients with TB/HIV co-infection was 16 (6.4%) of which 9 (3.6%) were males and 7 (2.8%) were females. The computed chi-square statistics indicated that there was no association between sex and TB / HIV co-infection. The age-frequency distribution of *M. tuberculosis* and HIV infections are shown in Table 3. The highest rate of infection for *M. tuberculosis* and HIV was 25 (10%) and 19 (7.6%) respectively, in the age group 26 - 35 years. The least rate of prevalence for TB infection, 2 (0.8%) was in over 56 years age group and for HIV infection, 4 (1.6%), was in 46 - 55 years age groups. There was no significant association between age and TB /HIV co-infection. However, there was significant association between age and HIV infection. Table 4 showed that 32 (12.8%) of patients with *M. tuberculosis* infection had severe anaemia (PCV 26 - 35%) and 5 (2.0%) had normal value (PCV > 36%). Again, 18 (7.2%) with HIV infection had severe anaemia (PCV 20 -25%), 13 (5.2%) moderate anaemia (PCV 26 - 35%) and 4 (1.6%) had normal value (PCV > 36%). Positive significance exist between anemia and *M. tuberculosis* and HIV infections ($p < 0.01$). Table 5 showed the results of the cross - tabulation between *M. tuberculosis*, Human HIV and erythrocyte sedimentation rate (ESR). It showed that none of the patients with *M. tuberculosis* and HIV infections had normal erythrocyte sedimentation rate (3 - 8 mm / hr). There was significant ($p < 0.01$) positive association between *M. tuberculosis*

and erythrocyte sedimentation rate (ESR) as well as HIV and erythrocyte sedimentation rate.

DISCUSSION

This study showed an overall prevalence of 21.6% *M. tuberculosis*, 14.0% HIV infections and 6.4% TB-HIV co-infection among patients in Umuahia, Abia State, South-East Nigeria. In Maiduguri, Northern Nigeria, Ukwandu (1998) reported a higher prevalence for *M. tuberculosis* infection (14.7%). Ibrahim et al. (2004) and Onifade and Dasekum (2000) in Lagos, South -west Nigeria also recorded lower prevalence of *M. tuberculosis* infection although they employed mantoux test for the diagnosis. In Abeokuta, south - western Nigeria, Ojo and Idowu (2005) recorded lower prevalence of HIV infections. The lower prevalence may be due to the fact that the individuals examined were pregnant woman attending antenatal clinics. Okodua et al. (2004) obtained a higher prevalence (9.6%) for TB-HIV co-infection in Edo State, Nigeria. Other states in Nigeria like Kano, Enugu, Borno, Plateau and Benue recorded prevalent rates of 12.0, 14.0, 27.0, 30.0 and 35.0% respectively, (Federal Ministry of Health, 2000). The relatively lower prevalent rate observed in this study could be due to the increase in awareness of HIV control measures, reduction in the number of sex partners, increase in the use of condoms as well as increased availability of antiretroviral drugs. In Table 2 the males have a higher TB-HIV prevalence rate 3.6% than females (2.8%). This higher rate could be due to higher exposure to HIV infection, which inadvertently predisposed the males to TB disease. HIV infection has been recognized as the most significant risk factor for progression of latent *M. tuberculosis* to active TB disease. The results of this study show that there was no significant difference ($P = 0.01$) between the sexes for both *M. tuberculosis* and HIV infections. This supports the report of other workers such as Sukutu et al. (1992) and Ibrahim et al. (2004).

Although, the highest rate of infections was among the age group 26 - 35 years, there was no significant difference between the age groups for *M. tuberculosis* and HIV infections in this study. This is in agreement with the previous work of Lawn and Achaempong (1999). This may be attributed to the fact that HIV infection is mainly transmitted sexually and the sexual desire is probably highest in the age range 25 - 35 year. The lowest rate of infections in the age groups 46 years and above may be due to diminished social habits and reduced sexual urge. *M. tuberculosis* significantly caused more anaemia (PCV 20 - 25%) compared to HIV infection. In the report of Campbell et al. (1994), which is similar to this study, HIV caused more anaemia. *M. tuberculosis* and HIV infections significantly caused extreme increase of erythrocyte sedimentation rate (ESR). Mafiana et al.

Table 2. Prevalence of *M. tuberculosis* and HIV infections by sex.

Sex	No. of TB +ve (%)	No. of TB -ve (%)	No. of HIV +ve (%)	No. of HIV -ve (%)	No. of TB / HIV +ve (%)	No. of TB / HIV -ve (%)	Total (%)
Male	31 (12.4)	96 (38.4)	18 (7.2)	109 (43.6)	9 (3.6)	118 (47.2)	127 (50.8)
Female	23 (9.2)	100 (40.0)	17 (6.8)	106 (42.4)	7 (2.8)	116 (46.4)	123 (49.2)
Total	54 (21.6)	196 (78.4)	35 (14.0)	215 (86.0)	16 (6.4)	234 (93.6)	250 (100)

Table 3. Age-frequency distribution of *M. tuberculosis* and HIV infections.

Age group (year)	TB Infections		HIV Infections		Row Total (%)
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
16-25	12 (4.8)	55(22)	6 (2.4)	61 (24.4)	67 (26.8)
26-35	25 (10)	49(19.5)	19 (7.6)	55 (22.0)	743 (29.6)
36-45	10 (4)	48 (19.2)	6 (2.4)	52 (20.8)	58 (23.2)
46-55	5 (2)	24 (9.6)	45 (1.6)	25 (10.0)	29 (11.6)
>56	2 (0.8)	20 (8)	0 (0)	22 (8.8)	22 (8.8)
Total	54 (21.6)	196 (78.3)	35 (14)	215 (86.0)	250 (100.0)

Table 4. Haematocrit level (PCV) in relation to *M. tuberculosis* and HIV infections.

Haematocrit (PCV)	TB Infection		HIV Infection		Row total(%)
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
20-25	32 (12.8)	35 (14.0)	18 (7.2)	49 (19.6)	67 (26.8)
26-35	17 (6.8)	70 (28.0)	13 (5.2)	74 (29.6)	87 (34.8)
> 36	5 (2.0)	91 (36.4)	4(1.6)	92 (36.8)	96 (38.4)
Total	54 (21.6)	196 (78.4)	35 (14.0)	215 (86)	250(100.0)

Table 5. Frequency distribution of erythrocyte sedimentation rate (ESR) in relation to *M. tuberculosis* and HIV infections.

ESR (mm / hr)	TB Infection		HIV Infection		Total
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
3 - 8	0 (0)	121 (48.4)	0 (0)	121 (48.4)	121 (48.4)
9 - 50	8 (3.2)	11 (4.4)	8 (3.2)	11 (4.4)	19 (7.6)
51-100	14 (5.6)	40 (16.0)	24 (9.6)	30 (12.0)	54 (21.6)
101-150	32 (12.8)	24 (9.6)	3 (1.2)	53 (21.2)	56 (22.4)
Total	54 (21.6)	196 (78.4)	35 (14.0)	215 (86.0)	250 (100.0)

(2007) supports this finding. The high prevalence of *M. tuberculosis* infection (21.6%) observed in this study as compared with the low rate of TB-HIV coinfection (6.4%) revealed that the resurgence of TB in Abia State may not be entirely due to HIV infection, although, the effect of HIV in the reactivation of TB cannot be ruled out. The upsurge of tuberculosis (TB) could be due to low standard of living, which inadvertently kept the immune status of the people at a low level, thereby increasing the

risk of developing tuberculosis (TB) faster. Low immunity has been found to predispose people to tuberculosis disease.

This study has been able to establish the prevalence of *M. tuberculosis* and HIV infections as well as that of TB-HIV co-infections amongst patients who attended clinics during the monitoring period. The infections were observed to affect mostly the economically and supportive group of the society. The overall prevalent

rate(s) in this study sounds a note of warning that more enlightenment campaigns should be carried out in the state to help curtail an upward trend of the infection. The control of HIV and tuberculosis therefore, should involve all facets of the government and all the various health professions.

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