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Detection of *Mycobacterium tuberculosis* among HIV seropositive patients attending Infectious Diseases Hospital (IDH), Kano, Nigeria

D. W. Taura[1]*, S. Rabiu[1], S. Idris[2] and Y. Mohammed[3]

[1]Department of Biological Sciences, Bayero University, Kano, Nigeria
[2]Department of Pathology, Infectious Diseases Hospital, Kano, Nigeria
[3]TB/HIV Unit, Infectious Diseases Hospital, Kano, Nigeria

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ABSTRACT: The study was conducted to determine the prevalence of the pulmonary tuberculosis (PTB) among the seropositive HIV patients and also the distribution of all PTB/HIV co-infection between May and August 2006. A total of 350 patients were screened, out of which 140 were PTB/HIV seropositive (40%) in which, 49(35%) were PTB smear-positive while 91 (65%) were found to be smear negative. Observations have shown that, prevalence rate of (21.4%), 30 were females, having the highest prevalence, while (13.6%), 19 were males. However, distribution based on occupational status indicated that, housewives (28.6%), 14 with highest prevalence rate while students (6.1%), 3 have the lowest. The results had shown that, age group between 35 – 39 years which was (9.29%) has the highest prevalence than 50 – 54 years which was the lowest (1.43%) statistical analysis using chi-square test have shown that there is no significant difference at 5%(df) level of significance.

Key Words: Pulmonary Tuberculosis; *Mycobacterium, Tuberculosis*, Infectious diseases; HIV Seropositive Patients.

Introduction

Human immunodeficiency virus (HIV) infection is major risk factors for the development of TB. The increase in reported cases of TB since the mid 1980s is attributed, in part to TB occurring in persons, infected with HIV, the virus that causes AIDS. AIDS robs the body of its natural ability to fight infection, making people with AIDS more likely to develop TB (Lung, 2005). TB and other mycobacterioses are well recognized complications of immunosuppression. In the 1980s the epidemic of HIV infection and its resulting immunosuppression in large number of persons have increased the incidence of mycobacterial diseases. Disseminated *Mycobacterium avium* complex (MAC) disease has become an important medical problem, MAC is the most common mycobacterial species isolated from persons with acquired immunodeficiency syndrome(AIDS).HIV infection appears to be an important risk factor for TB. Morever, TB is one of the few respiratory diseases occurring in HIV-infected persons that is transmissible, curable and preventable (Advisory committee for the Elimination of Tuberculosis 2000).

*To whom correspondence should be addressed.

TB is contagious disease that kills around 2million people each year one third of the world's population is currently infected with tuberculosis and someone is newly infected every few seconds (Avert, 2006).
Relationship between TB and HIV, TB is the leading cause of death among HIV infected people, WHO estimates that TB accounts up to third of AID death worldwide (joint HIV|TB intervention 2005). When someone is infected with TB likelihood of them become sick with the disease many times if they are also HIV positive (Avert 2005).

The contribution of HIV- related TB morbidity to total national TB morbidity is not precisely known, but HIV infection appears to have had a substantial imfact in some areas. Matching reported TB cases with the AIDS case registries in 43 states and 11 localities reveals that 4% of AIDS cases appear on the TB registries (Advisory committee for the Elimination of tuberculosis) (ACET, 2000)
There has been an upsurge in the prevalence of TB in both the developed and developing nations. African continent is the worst hit. Sub-Saharan Africa record the highest incidence of TB in 1985 with an estimate of 1.25million new cases that year. (Salami and Oluboyo, 2002)

Nigeria the most populous African country could not be exempt, but there is no formal national survey on TB to determine the true prevalence of the disease in the country. However, report from different parts of the country have long described TB to be endemic nationwide and the pulmonary form has been established to be the commonest. (Salami and Oluboyo, 2002). In another cohort of methadone-maintenance clients with documented positive tuberculosis skin test reactions, 14% of HIV-infected persons and none of the HIV –negative clients developed TB during a 2-years period. In Kinshasha, Zaire, a study of 500 decedents who were serologically tested postmortem showed that 16% of HIV-infected persons and 2% of HIV-negative persons had TB diagnosed infected persons and 2% of HIV-negative persons had TB diagnosed *ante mortem* by smear. (ACET,2000)

An association between TB and AIDS is particularly striking among groups with a high prevalence of both tuberculous and HIV infections, e.g. intravenous-drug users (IVDUs) and Haitians. However, HIV-related TB is not restricted to IVDUs and Haitians. It has been reported in homosexual and bisexual men and sexual contacts of bisexual men and in one person with transfusion associated AIDS. Demographically, minority populations in some areas have been at particular risk of HIV-associated TB. Detailed demographic information obtained from registry matching in New York city, Florida and New Jersey, revealed that blacks and Hispanics accounted for 80%, 90% and 100%, respectively, of the TB/HIV cases. (ACET, 2000)

Between 1990 and 2005, TB incidence rates tripled in African countries with high prevalence. Rates of TB are now rising across Africa by 3-4% annually. In 2004 an estimated 14million people worldwide were living with dual HIV/TB infections of whom 70% were African (Avert, 2006).

The largest number of TB cases occurs in the south-East Asia region, which in 2003 accounted for more than 3million cases (more than a third of the global total). However, the estimated incidence per capita in sub-saharan Africa is nearly twice that of south-East Asia, at 345 cases per 100,000 populations in 2003. Also, the countries of Eastern Europe are facing a growing epidemic, there were over 143,000 estimated cases in Russia alone in 2003 (Avert, 2006).

Materials and Methods

Study Area

The research work was carried out amongst HIV seropositive patients with pulmonary tuberculosis attending Infections Diseases Hospital (IDH), Kano. It is a reference hospital in the state where people from various parts of the state and of various occupations attends. It serves as a Federal Tertiary Health Center for diagnosis and treatment of HIV and tuberculosis.

Sample Collection

A total of 350 patients wire recruited for the study. Blood sample was collected and screened for HIV, out of which 140 were HIV seropositive. Sputum samples were collected from this patients for further analysis as employed by Baker *et al* (1998) and Fujiki (1998).

HIV SCREENING

Principle of the Test

The assay employs a unique combination of specific antibody binding protein, which is conjugated to colloidal gold dye particles and HIV 1/2 antigens, which are bound to the membrane solid phase. The sample is applied to the sample (S) well followed by additional of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the Test (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the Test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the Control (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

Procedure

A 5µL of the patients blood sample was transfered into the sample pad at the center of the sample well, which then followed by 3 drops of buffer slowly drop wise into the well.
The result was then read 10 minutes after the addition of the buffer solution.

COLLECTION OF SPUTUM SAMPLES

Sputum collection was done in the open air with the patient facing away from the wind and also away from others, during expectoration (Fujiki, 1998).

Principle of the Test

Mycobacteria have the ability to resist decolorisation in the present of a weak mineral acid after staining with arylmethane dye. In ZN staining method, carbol-fuchsin, combined with phenol binds, to the mycolic acid in the mycobacterial cell wall. After staining acid alcohol removes the red dye from the background cells, tissues, fibres and all other organisms in the smear except the mycobacteria, which retain the dye. Methylene blue is added as a counter stain. The acid fast bacilli will stain red and the non-acid fast bacteria including the background, will stain blue.

Procedure for making Smear and Ziehl's Neelsen Staining

- Slides were numbered accordingly
- Three sputum samples are usually collected within 2 days for new diagnoses, while two samples for follow-up patients
- Sputum was smeared using one loopful of the sputum from each container
- The smear was allowed to dry at room temperature
- The slides were then fixed by passing through flame about 2-3 seconds
- The slides, then stained by pouring carbol-fuchsin solution to cover the whole surface of the slide and heated for 5 minutes till steam rises up but not allow to boil.
- The slides were tilted and then washed with water and decolourized with 3% acid alcohol.
- The slides were washed with water again and tilted to drain excess water.
- Counter-staining was done with 0.1% methylene blue for 10 seconds, and washed with water.
- The slides were then placed on the slide rack and allow to dry
- The smear were examined under x100 objective

Results

Three hundred and fifty (350) patients were screened for HIV infection, in which 140 (40%) were found HIV seropositive, and 49(35%) patients out of the 140 were diagnosed Acid Fast Bacilli (AFB) or PTB positive, i.e. they were PTB/HIV co-infected.

Table 1, shows the age distributions of HIV seropositive patients diagnosed with pulmonary tuberculosis (PTB). The age range between 35 – 39 have the highest rate of PTB, with prevalence rate of 9.29%, and about 1.43% was found to be the lowest prevalence rate among age range between 50 – 54 years. The age distribution of PTB/HIV co-infected represented graphically as shown in Fig 1. (Appendix)

Table 1: Age distribution of PTB positive patients among HIV seropositive.

AGE GROUP	TOTAL NO. OF HIV POSITIVE PATIENTS	NO. OF PTB/HIV CO-INFECTED PATIENTS	PREVALENCE (%) OF PTB/HIV CO-INFECTED PATIENTS	NO. OF NON PTB/HIV CO-INFECTED PATIENTS	(%) OF NON PTB/HIV CO-INFECTED PATIENTS
15 – 19	17	3	2.14	14	10.00
20 – 24	14	4	2.86	10	7.14
25 – 29	13	7	5.00	6	4.29
30 – 34	26	10	7.14	16	11.43
35 – 39	27	13	9.29	14	10.00
40 – 44	21	7	5.00	14	10.00
45 – 49	11	3	2.14	8	5.71
50 – 54	11	2	1.43	9	6.43
TOTAL	***140***	***49***	***35***	***91***	***65***

Table 2, indicates that the rate of prevalence of PTB/HIV co-infected by occupational status. Housewives, drivers and traders have the highest rate of prevalence of infections, and 28.6%, 22.4% and 14.3% respectively. Students lowest prevalence with 6.1% while others accounted for 20.4%. The pie chart showing the PTB/HIV co-infected b occupational status was shown in Fig 2 (appendix).

TABLE 2: PTB/HIV co-infected by occupation status

Occupation	No. of PTB/HIV patients	Prevalence(%)
Housewives	14	28.6
Drivers	11	22.4
Traders	7	14.3
Students	3	6.1
Farmers	4	8.2
Others	10	20.4
TOTAL	***49***	***100***

Observations have shown that female patients have the highest incidence rate of prevalence of PTB/HIV co-infection, than males with 21.4% and 13.6% respectively (Table 3).

Table 3: PTB/HIV co-infection in relation to sex.

Sex	PTB positive	PTB negative	Total	Prevalence(%)
Female	30	47	77	21.4
Male	19	44	63	13.6
TOTAL	**49**	**91**	**140**	**35**

Table 4, shows a comparative analysis of the X^2 test among PTB positive and PTB negative in relation to sex at 5% (P>0.05) in which the disease has no any relationship to sex.Therefore, it can affect both sexes

Table 4: A comparative analysis of X^2 test among PTB positive and PTB negative patients in relation to sex at 5%(df).

sex	PTB positve	PTB negative	Total
Female	30	47	77
Male	19	44	63
TOTAL	**49**	**91**	**140**

The calculated value of X^2 is 1.18, while the table value is 3.81 at degree of freedom (df) 5% level of significance since the calculated value is less than the table value X^2 distribution, therefore, on the basis of significant difference, we agree with the null hypothesis.

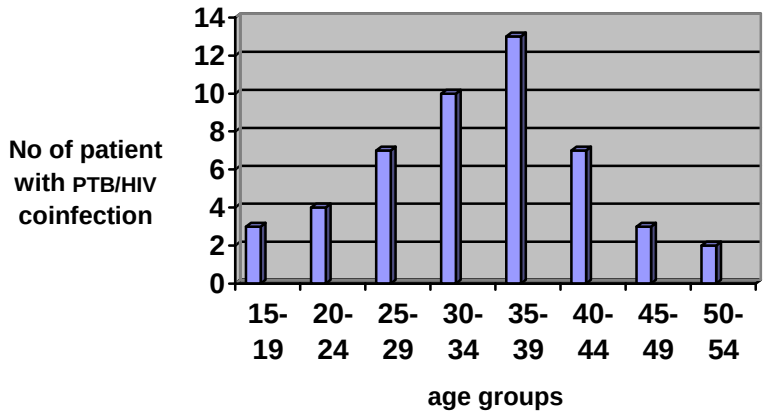

FIG 1:Graphic representation of age distribution among patients with HIV/PTB co-infection

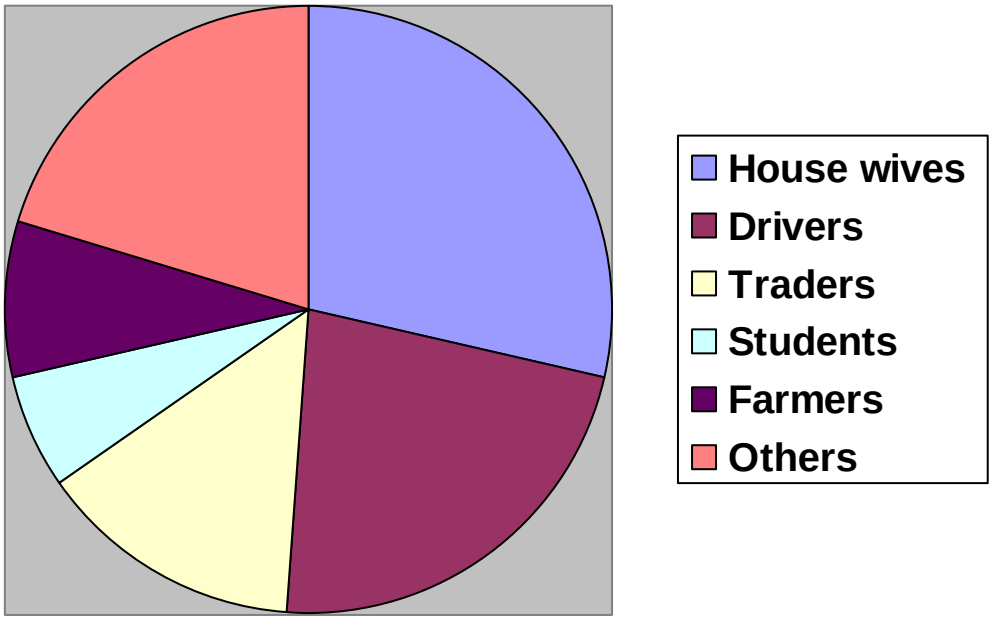

FIG 2: A pie chart showing the occupational status of PTB/HIV co infected patients

Discussion

A quarter of a million cases of TB was estimated to be present in Nigeria about a decade ago. The situation may have since hanged as reports from different parts of the country depict an increase in the prevalence of tuberculosis (Salami and Olubayo, 2002). A prevalence rate of 9.2% was observed by Salami and Olubayo. A prevalence rate of 14% was observed in this study. More than 75% of the affect patients were between 25 – 45 years.

In HIV infected patients, bacterial lower respiratory tract infections are the most frequent respiratory diseases. They are frequently the first clinical manifestation of HIV infection (Mayaud, 2002).

The results obtained have shown that the prevalence rate was 14% for PTB/HIV coinfection out of the 350 patients recruited for the research studies; which inclined with report of ACET, 2000. 140 (40%) patients were screened as HIV seropositive out of which 49 (35%) were PTB/HIV coninfected.

The observations made based on the age groups indicated that the incidence of PTB/HIV coinfections, have the highest rate of prevalence between 35 – 39 (9.29%) years, and thus has a great effects on youth development. This has shown an increased in the prevalence of tuberculosis.

Salami and Olubayo (2002) recorded a prevalence rate of 23% PTB/HIV coinfection amongst house wives,12% drivers, 25% traders ,4% farmers and 3%students, which inlined with my findings where by house wives (28.6%) appeared with the highest prevalence rate.

The high risk of infection was increased by certain factors, which include, malnutrition, illiteracy, poverty, overcrowding, poor knowledge about the disease , poor access to the center for HIV/PTB diagnosis this is common among rural dwellers, especially those living within scattered villages (huts) and nomadic herdsmen. This is similar to the report by JEETS, 2004 and Salami and Oluboyo (2002). In

addition to the above, lack of public enlightenment and awareness about the disease increase the rate of spreads to the society.

In the study, the results have shown that the highest rate of prevalence of PTB/HIV coinfection in relation to sex was among females with (21.4%), then males (13.6%). This is mainly affected females during their productive years.

The drugs of choice for treatments of this infection, includes Isoniazid, Rifampin, Ethambutol, Pyrazinamide, Streptomycin etc.

Conclusion

Based on my findings, it can be concluded that HIV infection has influenced the rapid rate of increased and progression of TB in the society, by weakening the cell mediated immune response, and thus become vulnerable to attacks by *M. tuberculosis*, similar finding were made by Zaw, 1999 and ACET, 2000.

Recommendations

It is recommended that the society should improve their life style by keeping away from unhealthy environments i.e. keeping away from smoking, drinking alcohol and drug abuse.It is also recommended that health education about symptoms, treatment be vigorously monitored by all stake holders in the health sector to reduce delays, so that patients can present early for treatments.

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