Comparative Analysis of Ziehl-Neelsen and Genexpert Techniques for the Diagnosis of Tuberculosis in Human Immuno-Deficiency Virus Positive Patients in Benin City

Ochei Kingsley Chinedum[1], Adagbonyin Emwiomwan[2], Obeagu Emmanuel Ifeanyi[3*] and Aminu Babayi[1]

[1]Family Health International (FHI 360) Country Office, Garki-Abuja, Nigeria

[2]Central Hospital, Benin City, Edo State, Nigeria

[3]Diagnostic Laboratory Unit, Department of Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

*Corresponding author: Obeagu Emmanuel Ifeanyi, Diagnostic Laboratory Unit, Department of Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, Tel: +2348037369912; E-mail: emmanuelobeagu@yahoo.com

Abstract

Mycobacterium tuberculosis is the second most common infectious cause of death in adults worldwide (HIV is the most common). The human host serves as the natural reservoir for *M. tuberculosis*. The ability of the organism to efficiently establish latent infection has enabled it to spread to nearly one-third of individuals worldwide. Approximately eight million new cases of active TB disease occur each year, leading to about 1.7 million deaths. The disease incidence is magnified by the concurrent epidemic of human immunodeficiency virus (HIV) infection. A total of 362 sputum specimens from 181 presumptive tuberculosis suspects of patients living with Human Immuno-Deficiency virus were tested using direct smear staining technique of ZiehlNeelsen and GeneXpert for the detection of MTB/RIF. The performance of Xpert MTB/RIF technique was compared withZiehl-Neelsentechnique, out of the 181 persons, 148 were smear negative (81.8%) and 33 were smear positive (18.2%), while for the Genexpert MTB/RIF technique, 115 (63.5%) were MTB not detected (negative), 59 (32.6%) MTB detected, RIF resistance not detected and RIF resistance detected, 4 (2.2%) error and 3 (1.7%) in-complete. Thirty-three (33) patients (18.2%) were TB positive with both techniques, Twenty-six patients (14.4%) were GeneXpert/RIF positive but ZN staining technique negative and 110 patients (60.7%) were negative with both methods. The positivity rate for MTB detected for Genexpert technique in female was 32 (58.2%) and male 23 (39.0%), while ZN staining technique in female was 15 (45.5%), and male 18 (54.5%). From this study, it was observed that Genexpert technique detected more tuberculosis cases than Ziehl-Neelsen technique. Amongst people living with Human Immuno-Deficiency virus, female are more diagnosed of tuberculosis than male with age range of 25-49 years having the highest positive result and the least positive result in age range 15-19

years 4 (2.2%), male 3 (1.7%) and female 1 (0.5%). Age distribution shows age group 25-49 years old with the highest number 120 (66.7%), with male 45 (25.0%)and female 75 (41.7%), followed by age group 50 years old and above 36 (20.0%), male 11 (6.1%) and female 25 (13.9%), then followed by age group 7-14 years old 13 (7.2%), male 8 (4.4%) and female 5 (2.8%). Age group 20-24 years old had 7 (3.9%), male 5 (2.8%)and female 2 (1.1%) and the least, age group 15-19 years old 4 (2.2%), male 3 (1.7%) and female 1 (0.5%). The sensitivity and specificity ratio were 85% and 97%. The integration of molecular techniques such as Genexpert in the identification of *M. tuberculosis* complex is recommended as this can provide timely intervention in the diagnosis and treatment of tuberculosis.

Keywords: Comparative analysis of Ziehl-Neelsen and genexpert techniques; Tuberculosis, Human immunodeficiency virus; Benin-City

Introduction

Mycobacterium tuberculosis is the second most common infectious cause of death in adults worldwide (HIV is the most common). The human host serves as the natural reservoir for *M. tuberculosis*. The ability of the organism to efficiently establish latent infection has enabled it to spread to nearly one-third of individuals worldwide. Approximately eight million new cases of active TB disease occur each year, leading to about 1.7 million deaths. The disease incidence is magnified by the concurrent epidemic of human immunodeficiency virus (HIV) infection [1].

Human Immuno-Deficiency virus (HIV) associated with tuberculosis (TB) remains a major global public health challenge [2], with an estimated 1.4 million persons worldwide. TB is still the most common opportunistic infection and cause of death among people living with HIV [3]. People

who are co-infected with TB/HIV face a higher risk of severe illness and early death as a result of the interaction between the two diseases. Tuberculosis is a curable disease and HIV is treatable, too often people do not access services quickly enough, or do not have access to prevention, or are in other ways unable to get the help they need to stay healthy, live long, productive and happy lives. Further, there has been an increase in rates of drug resistant tuberculosis, including multi-drug (MDR-TB) and extensively drug resistant TB (XDRTB), which are difficult to treat and contribute to increased mortality, this is due to the poor performance of sputum smear microscopy in HIV-infected patients, newer diagnostic tests are urgently required that are not only sensitive and specific but easy to use in remote and resource-constrained settings (Mycobacterial culture is a sensitive but slow way to diagnose TB. To halt the disease's spread, it is essential that TB-particularly TB that is resistant to several treatment drugs (multidrug-resistant, or MDR, TB)-is diagnosed quickly. GeneXpert MTB/RIFwas endorsed by the WHO in December 2010 and is generally recognized as break-through in TB diagnostics, for the investigating of patients who might have TB, especially in regions where MDR TB and HIV infection are common.

The Xpert MTB/RIF Assay is a highly sensitive, specific and rapid method for diagnosing TB which has potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity. Its usefulness in detecting sputum smear and culture negative patients' needs further evaluation in high burden TB and HIV areas under programmatic health care settings to ascertain applicability, cost-effectiveness, robustness and local acceptance. Early diagnosis and management of TB is also critical to reduce TB transmission in communities and health care facilities, although ART can reduce the incidence of TB both at the individual and population level, PLWH on ART still have higher TB incidence rates and a higher risk of dying from TB.

Human immunodeficiency virus (HIV) associated with tuberculosis (TB) remains a major global public health challenge [2]. *Mycobacterium tuberculosis* remains the most common opportunistic infection (OI) and is the most common cause of death in HIV infected patients. Tuberculosis is the most common co-infection in HIV positive clients who are at increased risk of both reactivation of latent TB infection and acquisition of new TB infections. Tuberculosis is the most common opportunistic infection (OI) among HIV-infected individuals, and co-infected individuals are at high risk of death [4]. TB may occur at any stage of HIV disease and is frequently the first recognized presentation of underlying HIV infection [5,6]. As compared to people without HIV, people living with HIV (PLWH) have a 20-fold higher risk of developing TB [6] and the risk continues to increase as CD4 cell counts progressively decline [5]. Opportunistic infections are significant contributors of morbidity amongst HIV infected individuals. Many of these infections are preventable and successfully treatable provided an accurate diagnosis could be made. HIV/AIDS patients with opportunistic and other endemic infections may not have the typical symptoms path gnomic of a particular infection. So, laboratory diagnosis becomes important in

management of these patients. Accurate treatment will go a long way in alleviating the pain and sufferings of HIV infected with opportunistic infections. Co-infection is infection with more than one disease at the same time (e.g., TB/HIV co-infection). There are estimated to be over one million people worldwide who have TB and HIV co-infection. The burden of disease through TB/HIV co-infection is particularly high in sub-Saharan Africa. HIV accelerates the natural progression of latent TB to active disease by lowering cell-mediated immunity. It has been documented that an individual who is HIV positive and infected with latent TB is 30 times more likely to develop active TB than an individual with latent TB but HIV negative.

The HIV/AIDS pandemic has caused a resurgence of TB, resulting in increased morbidity and mortality worldwide. HIV and TB have a synergistic interaction; each accentuates progression of the other. As the degree of immune suppression increases, the risk of developing TB disease also increases. *Mycobacterium tuberculosis* remains the most common opportunistic infection (OI) and is the most common cause of death in HIV infected patients. HIV-infected individuals, who have an extraordinary high risk of developing clinical TB, give cause for serious concern as the implications are most serious. Tuberculosis (TB) and HIV epidemic in sub-Saharan Africa continues to pose enormous challenges to public health. South Africa alone has 1 million people currently receiving HIV antiretroviral treatment [7], the TB incidence is 941 per 100,000 individuals [8].

Aim and Objectives

The aim of this study was to analyze sputum of patients living with Human Immune Deficiency Virus (HIV) in Benin City for *Mycobacterium tuberculosis* infection. The objectives of this study were to determine the prevalence of:

- *Mycobacterium tuberculosis* infection among people living with Human Immune Deficiency Virus (HIV)
- Comparative analysis of two diagnostic methods for detection of *Mycobacterium tuberculosis*
- The need for current diagnostic method embracing.
- The importance of using the new diagnostic tool for TB diagnosis amongst PLHIV.
- To find out the sensitivity of Genexpert MTB/RIF Assay method over Ziehl-Neelsen method in diagnosis of Tuberculosis infection.

Materials and Methods

Study area

This study was conducted in Central Hospital, Benin city, Edo State.

Study subjects

The study subjects comprised of all clients that are HIV positive and who had been coughing for a period of two weeks

and above with or without blood-tinged sputum, night sweat, weight loss, fatigue but visiting the clinic for the first time and relapse. Both male and female patients of all ages except age 0-6 years were enrolled for the study.

Collection and handling of samples

Sputum samples from suspected clients with complains of cough that lasted for two weeks and above, night sweat, weight loss, fever and fatigue, and those with known smear positive Tb cases but on treatment visiting Central Hospital, Benin city, Edo State were collected into clean (sterile) wide-mouth universal containers. After health talk on the patient on how to produce the sample, wide mouth container with a leak tie prove was often given. The client then moved to a designated site where there is open air flow to cough and produce the thick phlegm from the bronchi into the container and covers it with the lid. The samples were then received into the lab for documentation as patient's name age and serial number were written on the specimen container. Two samples; spot and early morning were collected from clients depending on their stages either suspect or follow-up.

Sample processing: To every sputum sample collected, two slides were labeled and used; for direct smear and the staining technique was employed Ziehl-Neelsen. Wooden applicator sticks (disposable) were used to make sputum smears of 2 cm by 1 cm on each slide thin enough that we were able to read newsprint through it. All stained slides were examined microscopically by light Microscopy. Positive and negative control slides were prepared from known positive and negative sputum samples and stained alongside with study samples using the stain for quality control of reagents and the microscopist.

Samples staining procedure

Ziehl-Neelsen method: Upon air drying, the slides were heat fixed with flame from Bunsen burner while on the staining rack spacing one from another with a distance of one centimeter. The entire slides were flooded with strong 3% Carbol fuchsine and heated under to steam, avoiding boiling and ensuring that the stain did not dry up and allowed for 3-5 minutes. This was followed by washing in a running tap water there after we applied 3% acid alcohol to decolourise the primary stain for 2-3 minutes ensuring that smears were not over decolourised. Finally, the counter stain 1% methylene blue was added for 1 minute, and then washed in running tap water. The slides were then placed on a rack to dry before examination.

Genexpert method

The Xpert MTB/RIF assay is a hemi-nested real-time PCR method that amplifies the 81-bp region of the RIF-resistance-determining region of the *rpoB* gene, positions 507-533. A sample reagent buffer containing NaOH and isopropanol is added in a 2:1 ratio to the processed sputum ensuring a final volume of at least 2 ml. After 15 min of incubation with intermittent hand mixing, 2 ml of the liquefied inactivated

sample is added to the cartridge that contains the wash buffer, reagents for lyophilized DNA extraction and PCR amplification, and fluorescent detection probes (five for the *rpoB* gene and one for an internal control, *Bacillus globigii* spores). After the cartridge is placed in the instrument module, the automated processes include the following: specimen filtering, sonication to lyse the bacilli and internal control spores, released DNA collection and combination with the PCR reagents, amplification, target detection by five-color fluorescence of overlapping molecular beacon probes, and one-color fluorescence for the internal control. Results are automatically generated within 2 h and reported as M. tb-negative or -positive (with semi-quantification) and RIF sensitive or resistant. The former determination is based on the amplification of any two rpo gene regions, and the latter determination is based on a difference of >3.5 amplification cycles of any probe.

Results

The **Table 1** shows positive cases with Ziehl-Neelsen (ZN) technique in relation to gender. Out of 181 patients tested, the percentage of males tested was 40.3 and the prevalence rate among male is 24.7.

Table 1 Prevalence of tuberculosis using Ziehl-Neelsen in relation to age groups and gender in Benin city.

Age groups (years)	Males tested	Number positive (%)	Females tested	Number positive (%)
7-14	8	1 (3.0)	5	0 (0.0)
15-19	3	0 (0.0)	1	0 (0.0)
20-24	5	0 (0.0)	2	0 (0.0)
25-49	46	13 (39.4)	75	13 (39.4)
50 and above	11	4 (12.1)	25	2 (6.06)
Total	73	18	108	15

One (1) male (3.0%) within the age range of 7-14 years was positive for Ziehl-Neelsen, 13 (39.4%) for age range 25-49 years and 4 (12.1%) age range 50 years.' and above, on the other hand, the percentage of females tested as 59.6 and the prevalence rate among female is 13.8. 13 (39.4%) females within the age range of 25-49 years were positive for Ziehl-Neelsen and 2 (6.06%) within the age range of 50 years. and above.

Positive cases with MTB/RIF Genexpert in relation to gender are shown in **Table 2**. Out of 181 patients tested, the percentage of males tested was 40.3 and the prevalence rate among male is 34.2. 2 (3.4%) within the age range of 7-14 years, 19 (32.2%) for 25-49 years and 4 (6.8%) for 50 years. and above, on the other hand, the percentage of females tested was 59.7 and the prevalence rate is 31.5. Female patients positive with MTB/RIF Genexpert were 2 (3.4%) for the ages of 15-19 years, 1 (1.7%) for the ages of 20-24 years, 28 (47.5%)

for the ages of 25-49 years and 3 (5.1%) for the ages of 50 years and above.

Table 2 Prevalence of tuberculosis using Genexpert MTB/RIF in relation to age groups and gender in Benin city.

Age groups (years)	Males tested	Number positive (%)	Females tested	Number positive (%)
7-14	8	2 (3.4)	5	0 (0.0)
15-19	3	0 (0.0)	1	1 (1.7)
20-24	5	0 (0.0)	2	2 (3.4)
25-49	46	19 (32.2)	75	28 (47.5)
50 and above	11	4 (6.8)	25	3 (5.1)
Total	73	25	108	34

Table 3 shows there was a significant difference the use of GenexpertMTB/RIF and ZiehlNeelsen techniques in the diagnosis of Tuberculosis.

Table 3 Comparative analysis of Ziehl-Neelsen and Genexpert techniques for diagnosis of tuberculosis in Benin city.

95% Confidence interval				
Gender	Number Tested	Genexpert Positive (%)	Ziehl-Neelsen Positive (%)	Odd ratio
Males	73	25 (34.2)	18 (24.7)	0
Females	108	34 (31.5)	15 (13.9)	2
Total	181	59 (65.7)	33 (38.6)	-
P-value=0.0001 (significant)				

The result in **Table 4** above shows patients who tested negative to tuberculosis using Ziehl-Neelsen technique tested positive to MTB/RIF Genexpert technique. The table also shows age distribution in relation to positive cases for MTB/RIF Genexpert. A t-test was conducted to test whether there was a significant difference between detection of tuberculosis using AFB and MTB/RIF Genexpert techniques. It must be recalled that the study hypothesized that detection of tuberculosis with ZN is low when compared with Genexpert amongst people living with HIV/AIDS or detection of tuberculosis with ZN is not low when compared with Genexpert amongst people living with HIV/AIDS. The results for the t-test indicate that at 95% confidence level the P value was 0.00 for both methods. Since the calculated p-value is less than 0.05 the null hypothesis is significant. Therefore it was concluded that detection of tuberculosis with ZN is low when compared with Genexpert amongst people living with HIV/AIDS.

33 out of 33 ZN technique positive were Genexpert technique positive, while 26 out of 148 ZN techniques negative were detected positive by Genexpert technique. The difference in the number diagnosed by Genexpert is a source of public health concern to embracing this modern technique without consideration of the capital. One person undiagnosed

will infects 10-15 persons per year, the multiplying effect of the 36 undiagnosed by ZN technique has unquantified social, economic, societal effect national and globally.

Table 4 AFB Ziehl-Neelsen negative, MTB/RIF Genexpert positive.

Age group (years)	Number tested	Ziehl-Neelsen negative	MTB/RIF Genexpert Positive
7-14	13	0	1
15-19	4	0	0
20-24	7	0	0
25-49	121	0	21
50 and above	36	0	1
Total	181	0	23

Wastage of the first line drug, capital, adverse side effect such as audio-toxicity (partial or complete), nephrotic syndrome and death are avoided for Genexpert MTB detected and RIF resistant cases directly. The chain of transmission of those that would have been infected with tuberculosis by the undiagnosed RIF resistant cases with ZN technique can be promoted by the early and prompt detection of such by Genexpert technique.

The overall prevalence/incidence of tuberculosis among HIV patients in the study population was high 1 ratio 3. Co-infection with tuberculosis can be attributed to the immunocompromised state of the patient which causes the patient to loose defense against invading pathogens.

Discussion

Mycobacterium tuberculosis is the leading cause of death after HIV from infectious diseases [1,9]. The combination of HIV and *M. tuberculosis* infection increases the mortality rate resulting from infectious disease. In order to curb the menace due to *M. tuberculosis* there is need for speedy, accurate laboratory diagnosis which will eventually lead to adequate treatment. This study was aimed at carrying out comparative analysis of Ziehl-Neelsen and Genexpert techniques for the diagnosis of tuberculosis in patients living with Human Immuno-Deficiency Virus in Benin City. In Nigeria, the interaction between TB and HIV is a growing problem which is impacting adversely on the efforts to control both diseases and on the overall health system in the country. Several studies in Nigeria showed the association between HIV and TB. From the study, the prevalence of *M. tuberculosis* with Ziehl-Neelsen technique in selected patients living with HIV in Edo state of Nigeria was found to be 41.6%. The prevalence rate among male is 24.7, while among female is 13.8.

Although this result found no significant association of age (P>0.05) with the prevalence of TB in people living with HIV, there was general increase and decrease in the prevalence of tuberculosis as the age progresses with the age group between

25 and 49 recording the highest proportional distribution (39.4%). The possible explanation for the highest proportional distribution recorded for 25-49 age groups could be attributed to the weakening of the immune system [10]. Even in the absence of HIV, this is the age group in general population where reactivation of latent tuberculosis takes place It has been estimated that the diagnosis of active TB with a sputum-based assay with a sensitivity of 85% and specificity of 97% has the potential to save more than 400,000 lives per year. But in this work the finding do not concord and the improvement of smear microscopy services remains necessary to increase patients' access to treatment. Furthermore, smear microscopy is known to be less sensitive in HIV-associated tuberculosis, and interventions which could increase this sensitivity are needed.

This study shows that the prevalence rate among male is 34.2 and female is 31.5. This study agrees with a similar study carried out in a primary health care clinic in Johannesburg, South Africa, where Among HIV+ individuals, the sensitivity of the Xpert MTB/RIF test was 84% (69%–93%), the reported accuracy of Xpert MTB/RIF for TB diagnosis-85% sensitivity and 97% specificity.

The Xpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing AFB smear microscopy and other molecular methodologies in an HIV- and TB-endemic region. Its place in the clinical diagnostic algorithm in national health programs needs exploration. It was suggested that Xpert MTB/RIF may provide a more accurate rapid diagnosis of TB than smear microscopy and other currently available NAAT tests in regions where HIV and TB are endemic (i.e., always present). Indeed, the reported accuracy of Xpert MTB/RIF for TB diagnosis-85% sensitivity and 97% specificity-has the potential to save more than 400,000 lives per year.

M. tuberculosis is the leading cause of death after HIV from infectious diseases. The combination of HIV and *M. tuberculosis* infection increases the mortality rate due to infectious disease. In order to curb the menace due to *M. tuberculosis* there is need for speedy, accurate laboratory diagnosis which will eventually lead to adequate treatment. In Nigeria, the interaction between TB and HIV is a growing problem which is impacting adversely on the efforts to control both diseases and on the overall health system in the country. Several studies in Nigeria showed the association between HIV and TB. The prevalence of *M. tuberculosis* in some selected people living with HIV in Edo state of Nigeria was found to be 62.1% with Genexpert as compared to 41.6% by Microscopy technique. Xpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing AFB smear microscopy and other molecular methodologies in an HIV- and TB-endemic region. To the best of our knowledge this is the first study to report tuberculosis infection using Genexpert technique in Benin City [11-16].

Formal identification is very important for epidemiological purposes and also it can also give a clue to the way the infection should be treated if antiTB drug susceptibility testing is not carried out on the isolates. Drug susceptibility testing

procedure for mycobacterial infection can only be best handled in reference laboratory. With the advent of several molecular biological techniques in the last 2 decades, this part can be handled with a laboratory that is well funded in developing countries.

The prevalence of *M. tuberculosis* complex on people living with HIV examined reveals that patients who were negative to acid alcohol fast bacilli test were found positive to MTB/RIF GENEXPERT test. This could be due to the ability of MTB/RIF GENEXPERT to detect very low numbers of bacilli compared to acid alcohol fast bacilli which may require higher number of bacilli. MTB/RIF is a cartridge-based, automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification technique (NAAT). There was a significant difference between both test results P<0.001.

Conclusion

Comparative analysis of presumptive tuberculosis amongst people living with human immuno-deficiency virus (PLHIV) in Central Hospital Benin-City. The usefulness of microscopy (Ziehl-neelsen) and molecular diagnosis (Genexpert) in laboratory diagnosis of tuberculosis was compared. The integration of molecular techniques such as Genexpert in the final identification of *M. tuberculosis* complex/*M. tuberculosis* is recommended as this can provide timely intervention in the diagnosis of tuberculosis and treatment. Identification of suspected *M. tuberculosis* from culture is a prelude to studying the molecular epidemiology of this organism.

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