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A Comparative Study between AFB Smear Microscopy (Ziehl-Nelsen) and Gene-Expert Technique in the Diagnosis of Tuberculosis

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Abstract **Original Research Article**

611 samples were collected and tested for AFB using the Zeihl-Neelsen technique. Of the 611 samples tested, 525 were negative with Ziehl-Neelsen AFB smear microscopy. The 525 negative samples were tested using the genexpert technique. 53 out of the 525 samples were positive with genexpert with a percentage positivity rate of 10.1%. The data analysis was done using the student's t-test. There was a significant difference between the sensitivity of Ziehl-neelsen technique when compared with genexpert technique ($t= 3.32$; $p< 0.05$). Hence, there was a demonstrated increase in laboratory based TB detection using Xpert MTB/Rif compared to smear microscopy (ziehl-neelsen).

Keywords: Smear Microscopy, Comparative Study, Gene-Expert.

INTRODUCTION

Tuberculosis (TB) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*. The m. tuberculosis complex (MTBC) includes four other TB-causing mycobacteria: M. bovis, M. africanum, M. Canetti, and M. microti [1]. M africanum is not widespread, but it is a significant cause of tuberculosis in parts of Africa. In Nigeria, the majority of TB infections are caused by M. tuberculosis followed by M. africanus and M. bovis [2]. M. bovis was once a common cause of tuberculosis, but the introduction of pasteurized milk has largely eliminated this as a public health problem in developed countries [3]. TB primarily affects the lungs, but it can also affect organs in the central nervous system, lymphatic system and circulatory system among others. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air.

Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. The disease was called 'consumption' in the past because of the way it would consume from within anyone who became infected. When a person becomes infected with tuberculosis, the bacteria in the lungs multiply and cause pneumonia along with chest pain, coughing up blood, and a prolonged cough. In addition, lymph nodes near the heart and lungs become enlarged. As the TB tries to spread to other parts of the body, it is often interrupted by the body's immune system. The immune system forms scar tissue or fibrosis around the TB bacteria and this helps fight the infection and prevents the disease from spreading throughout the body and to other people. If the body's immune system is unable to fight TB or if the bacteria break through the scar tissue, the disease returns to an active state with pneumonia and damage to kidneys, bones, and the meaning that line the spinal cord and brain. TB is generally classified as being either latent or active. Latent TB occurs when the bacteria are present in the body, but this state is inactive and presents no symptoms. Latent TB is also not contagious. Active TB is contagious and is the

condition that can make one sick with symptoms. The classic symptoms of active TB infection are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss infection of other organs causes a wide range of symptoms. A number of factors make people more susceptible to TB infections. The most important risk factors globally is HIV; 13% of all TB cases are infected by the virus [4]. This is a particular problem in sub-Saharan Africa, where rate of HIV are high. Tuberculosis is closely linked to both overcrowding and malnutrition, making it one of the principal diseases of poverty. Those at high risk thus include: people who inject illicit drugs, inhabitants and employees of locales where vulnerable people gather (e.g. prisons and homeless shelters), medically underprivileged and resource-poor communities, high-risk ethnic minorities, children in close contact with high-risk ethnic minorities, children in close contact with high-risk category patients, and health care providers serving these clients [5]. Chronic lung disease is another significant risk factor-with silicosis increasing the risk about 30-fold [6]. Those who smoke cigarettes have nearly twice the risk of TB than non-smokers [7].

Other disease states can also increase the risk of developing tuberculosis, including alcoholism and diabetes mellitus (threefold increase). Certain medications, such as corticosteroids and infliximab (an anti- TNF monoclonal antibody) are becoming increasingly important risk factors, especially in the developed world. There is also a genetic susceptibility for which overall importance remains undefined. Tuberculosis is indeed a global burden. An understanding of the epidemiology of Mycobacterium tuberculosis is critical for effective control. More than 2 billion people (about one-third of the world population) are estimated to be infected with mycobacterium tuberculosis, with new infections occurring at a rate of about one per second [8]. However, not all infections with M. tuberculosis cause tuberculosis disease and many infections are asymptomatic. In 2007 there were an estimated 13.7 million chronic active cases globally [9].

While in 2010 there were 8.8 million new cases, and 1.45 million deaths, mostly in developing countries [5]. 0.35 Million of these deaths occur in those co-infected with HIV [5]. Tuberculosis is the second most common cause of death from infectious disease, after HIV [10]. The incidence of TB varies with age. In Africa, TB primarily affects adolescents and young adults [11]. However, in countries where TB has gone from high to low incidence, such as the United States, TB is mainly a disease of older people, or of the immunocompromised [3].

In high-incidence countries, TB control relies on passive case finding among individuals self-presenting to health care facilitates, followed by either diagnosis based on clinical symptoms or laboratory diagnosis using sputum smear microscopy and other techniques. Early diagnosis of tuberculosis and initiating optimal treatment would not only cure patient but will also curb the transmission of infection and disease to others in the community of the several distinct components of TB control programme, case finding remains the corner stone for effective control of the disease. There are two basic approaches for the diagnosis of tuberculosis. The direct approach which includes detection of mycobacteria or its products and the indirect approach include measurements of humoral and cellular responses of the host against tuberculosis. The diagnostic modalities should have certain desirable features like sensitivity, specificity, predictive value, reproducibility, speed, cost effectiveness, safety, simplicity and easy application for wider use. Ideally, the test should be quantitative at least in some measure, so that the infectiveness of the individual cases can be measured. Diagnostic algorithms must be geared towards the specific needs of the population, the resources available in the individual countries and also the epidemiologically, the countries can be grouped as non-epidemic or endemic. The diagnostic need in disease non-epidemic countries include identification of latent infection in high risk groups, diagnosis of patients in early phase of the disease, faster detection of outbreak and finding out patients with non-tuberculosis mycobacterial disease. The diagnostic needs in disease endemic countries include improved microscopy, use of liquid culture, chemical and physical detection of mycobacterial antigens, antigen capture, antibody detection, cellular immune recognition, nucleic acid amplification and phage assay. Extended treatment with multiple drugs in needed to effectively cure tuberculosis. The main reasons for this requirement are the hydrophobic cell envelope surrounding members of the Mycobacterium tuberculosis complex (MTBC) that serves as a permeability barrier to many compounds; the sequestered, non-replicating subpopulation of TB that is affected by drugs only when the cells reemerge from dormancy; and the drug target or drug-activating enzymes in TB that are altered by mutation and result in a population of drug-resistant cells [12]. The currently used four-drug 6-to9- month therapy with rifampin (RIF), isoniazid (INH), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM) is necessary to kill actively replicating TB, eliminate most persisting organisms, and inhibit the development of resistance. However, patients who are instructed to complete the extended regimen often stop taking the pills when they begin to feel better or are noncompliant due to the high pill burden or for other reasons [13].

Currently, Ziehl-Neelsen sputum smear microscopy is the simplest and most rapid test available to detect acid fast bacilli (AFB) in clinical specimen. Although it is simple and inexpensive with a specificity of almost 100%, it requires minimal infrastructure and equipment, detects patients most likely to transmit the disease and pose an infection risk, accessible to

majority of patients and monitors treatment success, it suffers from a low sensitivity ranging from 22-78% and high bacterial load (> 5000 AFB/ml) is required for detection [14]. So, some TB infections are missed. However, the genexpert technique is highly sensitive (99%) and requires about 150 AFB/ml) of sputum for detection.

Smear preparation

The frosted end of the slide was labelled with pencil .An applicator stick used to transfer a purulent or blood tinged portion of the specimen to the center of the slide and a thin smear was made using concentric circular movement. The smear was placed on a flat surface facing upwards and allowed to dry for one hour and then fixed by passing three times through a Bunsen flame.

Staining procedure

The slides were arranged on a staining rack with smear side facing up and flooded with 0.1% carbol fuchin. Bursen flame was passed under the slide until stain steams and then allowed to stain for 5 mins. The smears were washed with water and drained. Decolonization was followed using 3% acid alcohol for 3 mins. The smears were rinsed with water n, drained and counter stained with 0.1% methylene blue for 1 min. The smears were rinsed with water , drained and allowed to air dry, then examined microscopically using the oil immersion (x100)

Smear evaluation

Who/iuatld quantification scale

Ziehl –neelsen

Number of AFB	Number of fields	Report
No AFB in 100 fields	100	No AFB DETECTED
AFB in 100 fields	100	Record exact figure
AFB in 100 fields	100	1+
AFB in 100 fields	50	2+
AFB in 100 fields	20	3+

The gene expert technique

Principle

The principle of the test is based on the amplification of target nucleic acid sequences in the TB genome (using Real Time PCR). In a positive MTB rifampicin sensitive sample the target nucleic acid are annealed to 5 probes which are labelled with florescent dyes. The 6^{th} probe binds to the sample processing control (SPC) which consists of non-TB DNA and is already present in the cartridge. In rifampicin resistant samples one or more of the probes will not anneal to the target nucleic acid due to mutation. When all the probes are not annealed to the DNA, it indicates a negative sample for MTB. When the SPC control probe does not bind the test is invalid.

Sample preparation

The sputum sample was diluted with sample reagent (1:2). It was mixed vigorously and incubated for 10mins.

After incubation, it was mixed vigorously again and incubated for another 5mins.

Procedure

The cartridge was labeled with sample identification number and 2ml of mixed sample was transferred into the open port of the xpert MTB/RIF cartridge using Pasteur pipette. The cartridge barcode was scanned and inserted into the gene xpert machine and allowed for 2hours.

The result was read and interpreted.

RESULT

611 sputum samples were collected and tested using the ziehl-neelsen sputum smear microscopy. Of the 611 samples, 525 were negative for ziehl-neelsen AFB smear microscopy. The 525 negative samples were then tested using the genexpert technique. 53 out of the 525 samples were positive with genexpert with a percentage positivity of 10.1%.

Month	Total smear	Smear negative	Genexpert negative	Genexpert positive	% positivity
APRIL	180	154	134	20	13%
MAY	74	61	51	10	16.4%
JUNE	63	52	49	3	5.8%
JULY	96	81	75	6	7.4%
AUGUST	93	80	74	6	7.5%
SEPTEMBER	105	97	89	8	8.2%
TOTAL	611	525	472	53	10.1%

$t= 3.32$, $p < 0.05$ $df = 5$

In the month of April, 180 samples were tested using ziehl-neelsen technique, 154 samples were negative with ziehl-neelsen technique, 134 were negative with genexpert while 20 were positive with percentage positivity of 13%. In the month of May, 74 samples were tested using ziehl-neelsen technique, 61 samples were negative with ziehl-neelsen technique, 51 were negative with genexpert while 10 were positive with percentage positivity of 16.4%.

In the month of June, 63 samples were tested using ziehl-neelsen technique, 52 samples were negative with ziehl-neelsen technique, 49 were negative with genexpert while 3 were positive with percentage positivity of 5.8%.

In the month of July 96 samples were tested using ziehl-neelsen technique, 81 samples were negative with ziehl-neelsen technique, 75 were negative with genexpert while 6 were positive with percentage positivity of 7.4%.

In the month of august, 93 samples were tested using ziehl-neelsen technique, 80 samples were negative with ziehl-neelsen technique, 74 were negative with genexpert while 6 were positive with percentage positivity of 7.5%.

In the month of September, 105 samples were tested using ziehl-neelsen technique, 97 samples were negative with ziehl neelsen technique, 89 were negative with genexpert while 8 were positive with percentage positivity of 8.2%.

The data was analyzed using the student's t-test at 95% confidence limit with a mean and standard deviation 8.83 and 5.95 respectively. The difference was statistically significant ($t= 3.32$; $P < 0.05$)

DISCUSSION

In this study, a comparative test was carried out between ziehl-neelsen sputum smear microscopy and Xpert MTB/Rif technique. Using the Xpert MTB/RIF technique, the laboratory was able to diagnose an additional 53 cases of active TB among individuals who were smear negative on microscopy ; this corresponds with a positivity rate of 10.1% and this was statistically significant (table 1). The study supports the alternative hypothesis (Ha) which states that there is a significant difference between the sensitivity of ziehl-neelsen technique when compared with that of Xpert MTB/RIF technique.

This is in line with the study of who reported that Xpert MTB/RIF outperformed smear microscopy, established a diagnosis in a significant proportion of patients with smear negative TB and detected many highly likely TB cases missed in a study found that Xpert MTB/RIF sensitivity for smear positive, culture positive TB cases was very high (100%) and Xpert MTB/RIF sensitivity in smear negative, culture positive TB cases was lower (66.6%), two out of three cases and specificity for both 100%. Thus, the MTB/RIF assay has a sensitivity that approximately approaches that of culture.

Thus, drew attention that patients with smear-negative TB, can make use of these Xpert assay results to reduce the time to start off treatment from 56 days to 5 days.

In addition, reported that Xpert MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours.

Xpert can be used as an initial diagnostic test for TB detection and rifampicin resistance in patients suspected of having TB, MDR-TB or HIV-associated TB. Xpert may also be valuable as an add-on test following microscopy for patients who have previously been found to be smear negative and that Xpert MTB/RIF when used in replacing the conventional drug susceptibility, it can detect 94% of RIF resistant TB with high specificity of 98%.

Also pointed that the rates of untreated smear-negative culture positive TB decreased from 39.3% without Xpert to 14.7% using the assay to direct treatment initiation.

Concluded that Xpert MTB/RIF assay is a rapid, accurate point-of-care diagnostic test that is affordable and can be readily implemented in urgently needed conditions defined Xperts limit of detection by "the lowest number of colony forming units per sample that can be reproducibly distinguished from negative samples with 95% confidence. Despite microscopy being the diagnostics test most widely used worldwide, only 45% of TB cases that were notified in 2009 were sputum smear-positive, and these represented just 28% of the estimated total burden of incident disease globally.

In the past, these smear negative cases would initially be treated with broad spectrum antibiotics, followed up and re-assessed for TB if symptoms persisted.

There would be additional delays to TB treatment if these patients did not come back for a re-assessment or actually felt better with the initial treatment.

From a public health of view, a single case missed is a public health threat. The stop TB partnership of halving TB prevalence and death rate by 2015, compared with 1990 levels, thus paving way for the elimination of TB (defined as less than one case of

TB disease per one million population per year) by 2050 will not be achieved without enhanced case detection rate [4]. Early diagnosis of tuberculosis and initiating optimal treatment would not only enable cure of an individual patient but will also curb the transmission of infection and disease to others in the community.

The Xpert MTB/RIF technique does not only detect TB Infection but will also indicate rifampicin resistance which can give an idea to multiple drug resistance TB.

CONCLUSION

There was a demonstrated increase in laboratory based TB detection using Xpert MTB/RIF compared to smear microscopy (ziehl neelsen). Hence Xpert MTB/RIF is more sensitive than ziehl-neelsen smear in microscopy. Conclusively, the increased sensitivity in smear negative TB, along with high specificity of Xpert MTB/RIF mean that it may be used as the initial diagnostics test for TB detection in individuals suspected of having TB and MDR-TB. Xpert MTB/RIF may also be valuable as add on test following a negative smear microscopy result in patients suspected of having TB. In addition, the high sensitivity and specificity of Xpert MTB/RIF for RIF resistance detection mean that that it may be used as an initial diagnostic test for RIF resistance. These results can be considered as an initial step to use Xpert MTB/RIF to control the spread of TB and MDR-TB. Given the high mortality rate of undiagnosed TB especially in people living with HIV, this could save lives.

RECOMMENDATIONS

Based on this finding, it is recommended that every patient that is negative for sputum smear microscopy should be tested using the Xpert MTB/RIF technique. This is to ensure that no positive patient is lost to the community thereby posing a threat to public health.

Despite the fact that this technique is not cost effective and is not affordable to health centers in the rural communities, sputum samples can still be collected in such centers and sent to facilities who can afford it.

Also government and other NGOs can make the Xpert machine available in secondary and tertiary health institutions where individuals can access the test free of charge.`

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