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RESEARCH ARTICLE

Diagnosis of Pulmonary Tuberculosis in Resource Limited Setting of Rawalpindi

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Abstract:

Introduction:

Tuberculosis is an infectious disease with a high prevalence of about 9 million cases occurring annually. Ziehl Neelsen microscopy is the most widely used technique to detect Acid Fast Bacilli, but it is less sensitive. However, fluorescent microscopy is more helpful with simple diagnostic criteria. Gene Xpert® MTB/RIF assay is a rapid molecular assay that enables diagnosis of Tuberculosis with simultaneous detection of rifampicin resistance. Owing to this fact, we aimed at evaluating the diagnostic accuracy of Ziehl Neelsen microscopy, fluorescent microscopy and Xpert MTB/RIF keeping MTB culture (Mycobacterial Growth Indicator Tube) as a gold standard for the diagnosis of tuberculosis.

Methodology:

This study was carried out at a tertiary care hospital of Rawalpindi in the year 2016. Patients aged 18 to 70 years irrespective of gender with suspected TB based on history, clinical and radiological examination were included in the study. Respiratory clinical specimens including sputum, Broncho-Alveolar Lavage (BAL), and endobronchial washings were collected. Specimens were processed by MGIT (MTB culture), ZN microscopy, fluorescent microscopy and Gene Xpert MTB/RIF assay.

Results:

A total of 352 respiratory specimens were tested among which 160 (45%) samples were positive by culture. Out of culture positive samples, 158 samples (98.7%) were GeneXpert TB positive while 2 were negative. While only 49 (30.6%) were positive on ZN microscopy and 89 (55%) were positive on fluorescent microscopy. Out of the culture negative samples, 2 were positive with ZN microscopy, one was positive with fluorescent microscopy and 3 were positive on Gene Xpert. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and diagnostic accuracy of ZN Smear microscopy was 39%, 99.5%, 96%, 63% and 14.5% respectively. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of fluorescent smear microscopy was 55% and 99.5%, 98%, 72% and 79% respectively. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of Gene XPERT was 98% and 99%, 98%, 99% and 98% respectively.

Conclusion:

In countries like Pakistan where Tuberculosis is endemic, the diagnostic accuracy with highest sensitivity and specificity was Gene Xpert Polymerase Chain Reaction (PCR) MTB/RIF assay which can help in well-timed diagnosis of the disease.

Keywords: Pulmonary Tuberculosis, Fluorescent microscopy, Gene Xpert, Auramine phenol stain, WHO report, Positive predictive value.

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1. INTRODUCTION

Pulmonary Tuberculosis (TB) is a global health burden. According to the WHO report of 2015, there were 10.4 million reported cases of TB and 1.4 million people died because of the disease [1]. Pakistan is one of the six countries which accounts for 60% of the global TB burden. Timely and accurate diagnosis of tuberculosis remains pivotal for controlling this disease. Ziehl Neelsen (ZN) staining is still widely used in most parts of the world as a diagnostic tool; however, this method has low sensitivity and specificity. Fluorescent microscopy is more sensitive than Ziehl Neelsen staining [2, 3]. Cases of scanty bacilli which are unable to pick up on smear microscopy may lead to delay in diagnosis and subsequent treatment. Mycobacterial culture is considered to be the gold standard for diagnosis of TB; however, conventional method using Lowenstein Jensen media is time consuming [4, 5].

A variety of Nucleic Acid Amplification (NAA) methods have been developed for the rapid detection and identification of pulmonary and extra-pulmonary *Mycobacterium tuberculosis* [6].These techniques are both, fast and accurate but require costly equipment and technical expertise. Recently, the WHO has endorsed GeneXpert (Xpert® MTB/Rif assay) for the diagnosis of TB which aids in rapid identification of *Mycobacterium tuberculosis* and Rifampicin resistance related mutations simultaneously in an unprocessed sample. Diagnostic accuracy of GeneXpert for pulmonary TB is high and it is also useful to detect *M. tuberculosis* in samples with no or scanty bacilli [7, 8].

The rationale of our study was to revisit the diagnostic accuracy of ZN staining, Fluorescent staining and GeneXpert keeping culture (MGIT) as the gold standard for resource scarce settings.

2. MATERIALS AND METHODS

The study was conducted from January 2016 through December 2016 at Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan. Ethical approval was sought from Research Ethics Review Committee. After a written informed consent, patients aged 18 to 70 years irrespective of gender with suspected TB based on history, clinical and radiological examination were registered for the study. Respiratory clinical specimens including sputum, Broncho-Alveolar Lavage (BAL), and endobronchial washings were collected. Patients already diagnosed with pulmonary TB, repeat sample of the same patient, improperly collected samples and patients already on anti-TB treatment were excluded.

All respiratory specimens were processed by the standard N-acetyl-L-cysteine and sodium hydroxide method with final concentration of NaOH as 2%. Tube containing digested and decontaminated specimens was centrifuged (3000 × g) for 15-20 minutes after which the supernatant was discarded and deposit was used for ZN staining, fluorescent staining and culture.

2.1. ZN Staining

2-3 drops from specimen were placed on a glass slide to prepare a smear before it was inoculated into (Mycobacteria Growth Indicator Tube) MGIT 960 system. The smear was then placed into an oven at a temperature of 56ºC for about 5-6 minutes for drying, followed by the ZN staining. The number of Acid fast bacilli present was reported as; 1-9 bacilli/100 fields = 1+, 1-9 bacilli/10 fields = 2+, 1-9 bacilli/field = 3+ and more than 9 bacilli/field = 4+.

2.2. Culture

All the specimens after being digested and decontaminated processed along with MGIT growth supplement OADC (Oleic acid, Albumin, Dextrose, Catalase) and PANTA (Polymyxin-B, Amphotericin-B, Nalidixic acid, Trimethoprim and Azlocillin) were inoculated into MGIT 960 TB system (Becton Dickinson, Sparks, USA) after scanning bar code. All these specimens along with a positive control MGIT tube (containing ATCC 25177 *Mycobacterium tuberculosis*) and an un-inoculated negative control MGIT tube were incubated in MGIT 960 until the instrument showed a tube positive or negative. After a positive signal, the tube was removed from the MGIT 960 system after scanning bar code and ZN staining was done for the confirmation of the presence or absence of AFB. Mycobacteria Other Than Tuberculosis (MOTT) were checked by rapid immunochromatographic technique using "BD MGIT™ TBc Identification Test".

2.3. Fluorescent Microscopy

2-3 drops from the specimen were placed on a glass slide to prepare a smear before it was inoculated into MGIT 960

system. The smear was then placed into an oven at a temperature of 56ºC for about 5-6 minutes for drying, followed by the auramine phenol staining for 20 minutes, slide was rinsed with water followed by decolorisation by acid alcohol and counterstained with 0.1% potassium permanganate for 30 seconds. AFBs were visualized by using fluorescent microscope in 250 x magnification power. If a sample contained 1- 9 Acid Fast Bacilli (AFB) per 10 fields it was categorized as +1, if it contains 1–9 AFB/field this was categorized as 2+, if a sample contains 10-90 AFB/field it was be categorized as 3+, while if a sample contains >90 AFB/field it was be categorized as 4+.

2.4. Gene Xpert PCR

One milliliter (ml) of the remaining deposit of clinical sample was transferred to a screw-capped tube containing 2 ml of sample reagent at a ratio of 1:2; this reagent inactivated the sample with NaOH and isopropanol. The mixture was then incubated for 15 minutes at room temperature and mixed every five minutes until liquefied with no visible clumps. The mixture was transferred into the Xpert MTB/RIF cartridge using the sterile pipette provided until the meniscus was above the minimum mark. The Xpert MTB/RIF cartridge includes an internal control for sample processing (DNA extraction and for PCR presence inhibitors). The inoculated cartridge was placed into the GeneXpert instrument (GX). Results were available in less than two hours and interpreted by the GX system automatically.

3. RESULTS

A total of 352 respiratory specimens (202 sputum, 95 EB washings and 55 BAL samples) were tested. Mean age of the patients was 42.75±14.73 years. There were 258 (73.3%) male and 94(26.7%) female patients. Out of total 352 specimens, 192 specimens were negative by all 4 modalities. 160 samples were positive by culture including 97 sputum, 26 Endobrochial washings and 37 BAL specimens. Out of the 160 that were culture positive, 158 samples (98.7%) were GeneXpert TB positive while 2 were negative. Out of the 160 samples that were culture positive, only 49 (30.6%) were positive for ZN microscopy (10 specimens were detected as +1; 13 specimens were +2; and 26 specimens were +3 for AFB) and 89 (55%) specimens were positive by fluorescent microscopy. Out of the culture negative samples, 2 were positive for ZN microscopy, 1 was positive for fluorescent microscopy and 3 were positive on Gene Xpert. The specimen that was positive on fluorescent microscopy was also positive on Gene Xpert and ZN microscopy.

Sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) and diagnostic accuracy of ZN Smear microscopy was 39%, 99.5%, 96%, 63% and 14.5% respectively. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of fluorescent smear microscopy was 55% and 99.5%, 98%, 72% and 79% respectively. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of Gene XPERT was 98% and 99%, 98%, 99% and 98% respectively.

Sensitivity, specificity, Positive predictive value, Negative predictive value and agreement for ZN smear microscopy, Fluorescent smear microscopy and GeneXpert is shown in Table **1**.

Table 1. Sensitivity, specificity, PPV and NPV of different diagnostic modalities (n=352).

-	-	Culture		(%)				
-	-	Positive	Negative	Sensitivity	Specificity	PPV	NPV	Agreement
ZN Microscopy	Positive	49	02	39	99	96	63	14.5
	Negative	111	190					
Fluorescent Microscopy	Positive	89	1	55	99	98	72	79
	Negative	71	191					
Gene Xpert	Positive	158	03	98	99	98	99	98
	Negative	2	189					

PPV: Positive Predictive Value, NPV: Negative Predictive Value.

4. DISCUSSION

Early diagnosis and prompt treatment of TB plays a key role in breaking the chain of its transmission. The purpose of this study was to evaluate the diagnostic yield of different modalities in diagnosing tuberculosis in respiratory specimens and comparing there outcomes with MTB culture. Sensitivity of ZN microscopy depends on factors such as smearing technique, viewer experience and number of bacilli in the specimen. Unfortunately, microscopy is not a very sensitive tool for the diagnosis of tuberculosis [9, 10]. A study conducted in India concluded the sensitivity of ZN stain to be 55.5% and that of Fluorescent microscopy 71.85%. Specificity was 99.2% for both staining methods employed [11]. Sensitivity of ZN staining in our study was 39.5% whereas fluorescent stain was 55%, a lower sensitivity.

However, specificity was comparable to that of the former study. Our results for ZN microscopy were in complete concordance with the study done by Agarwal, *et al.* except our sensitivity was higher [5]. Our results for ZN microscopy were also similar to the study done by Khalil KF except that study had a very low negative predictive value [12].

In many developing countries where there are limited resources, the diagnosis of tuberculosis is based completely on microscopy of ZN stained smears. The fluorescent stain, auramine, however, has displayed better performance and is more sensitive than the ZN stain. Therefore, auramine may be a cost-effective step to improve the diagnosis of tuberculosis in settings where fluorescent microscope is available. Cattarmanchi, *et al.* reported the sensitivity and specificity of fluorescent microscopy to be 72% and 81% respectively. Our results are not in concordance to this study as we have a relatively lower value for the sensitivity and higher for the specificity [13]. Results for GeneXpert accuracy were in relative concordance with Sharma SK *et al.*, Barnard DA *et al.* and Reechaipchkul *et al.* [9, 10, 14]. Khalil KF *et al.* conducted a study to evaluate the efficacy of GeneXpert in Pakistan and results were comparable with our findings [12].

Fluorescent microscopy is a simple modality with better performance and high diagnostic yield compared with ZN microscopy, as shown in this study. Gene Xpert MTB/RIF was a rapid, sensitive, accurate and reliable method for diagnosis of TB along with rifampicin resistance in present study. Patients with false positive results may receive unnecessary anti-TB therapy. False positivity of Xpert MTB/RIF assays and microscopies may be attributed to the presence of dead MTB test samples particularly among previously treated patients. In a resource scarce country like Pakistan where the disease is endemic modalities like Gene Xpert MTB/RIF believed to be more beneficial in timely diagnosis of a highly contagious disease.

CONCLUSION

The results of our study conclude that fluorescent microscopy has better diagnostic accuracy in comparison to ZN staining and is cheaper as compared to PCR. But Gene Xpert PCR is a modality with very high sensitivity and specificity and can detect not only *Mycobacterium tuberculosis* but also rifampicin resistance simultaneously. In countries like Pakistan where the disease is endemic and where facilities of fluorescent microscopy and Gene Xpert PCR are available, combination of these two modalities can help in making a timely diagnosis and initiating timely treatment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was sought from Research Ethics Review Committee.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Informed consent were obtained.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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