

ORIGINAL ARTICLE**A COMPARATIVE STUDY OF GENE XPRT AND VISUAL SPUTUM MICROSCOPY OF TB CASES IN SIALKOT, PAKISTAN IN LAST ONE YEAR**

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<p>Affiliations</p> <p>1. Professor & Dean Research & Academics, Sialkot Medical College, Sialkot sahibzadadrSyed786@gmail.com</p> <p>2-6 MBBS students at Sialkot Medical College, Sialkot mominagazafi4thyear2023@smcs.com.pk sayedaseharabbas4thyear2023@smcs.com.pk emanawais4thyear2023@smcs.com.pk alisharafique4thyear2023@smcs.com.pk mianshamrozimran4thyear2023@smcs.com.pk</p> <p>7. Sonologist, Usman General Hospital Ugoke, Lecturer University of Sialkot. gillanisyed2112@gmail.com</p> <p>Corresponding Author: Dr. Syed Muhammad Shah Hussain Miran, Sonologist, Usman General Hospital Ugoke, Lecturer University of Sialkot Contact # 0333-8605380 Email: gillanisyed2112@gmail.com</p>	<p>Abstract:</p> <p>Objective: To compare the efficacy of Gene Xpert with visual microscopy of sputum.</p> <p>Methods: During 01 year April, 2022 to April, 2023, total 148 sputum samples were collected. Sputum samples were collected from each patient & on these samples Visual Microscopy & GeneXpert were performed.</p> <p>Results: Out of 148 patients only 13 were in age group of 10-18 years, while 28 were in 19-29 years, however 29 were of 30-40 years, 55 were of 41-60 years and 23 patients were in age group of >60 years, respectively. For the purpose of confirmation of TB, smear microscopy and GeneXpert were performed. Referring to microscopy, 8, 24, 21, 39 and 17 patients were reported positive from age groups of 10-18, 19-29, 30-40, 41-60 and >60, respectively. 5, 4, 8, 16 and 6 patients were reported negative with TB from age groups of 10-18, 19-29, 30-40, 41-60 and >60, respectively. All 48 selected samples were reported positive when GeneXpert technique is used.</p> <p>Conclusion: GeneXpert has become superior to AFB culture in the diagnosis of TB</p> <p>Keywords: Gold Standard, Microscopy, GeneXpert, ZN staining</p> <p>Cite this Article as: Syed S.S.M., Qazafi M., Abbas S.S., Awais E. Rafique A., Imran M.S., Hussain S.S.; <i>A Comparative Study of Gene Xpert and Visual Microscopy of Sputum of Pulmonology and TB Cases in Sialkot, Pakistan in One Year Sialkot, Pakistan In One Year. SIAL J Med. Sci. 2023 V-2 (Issue-01):29-35</i></p>
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Introduction

A leading cause of death from a single infectious agent, tuberculosis (TB) has become a global public health issue which results in high rate of increase in resistant to treatment cases. To stop the emergence of medication resistance strains, it is essential to give prompt and effective therapy. For efficient case management, this necessitates the availability of quick and trustworthy Point of Care (POC) diagnostic techniques. Clinical, immunological, microscopy, radiography, and bacterial culture are frequently used techniques for TB screening and diagnosis. Additionally, to

diagnose and characterize TB, there are new developments in molecular diagnostic techniques, such as GeneXpert and whole genome sequencing (WGS), have been used. These techniques can both detect *Mycobacterium tuberculosis* (MTB) and the mutation(s) linked to commonly prescribed anti-TB medications. Here, we examine the application of current diagnostic approaches, from traditional to recently adopted NGS (Next Generation Sequencing Techniques) used to diagnose MTB from a medical standpoint.

Tuberculosis is a contagious, potentially deadly disease caused by *Mycobacterium*

tuberculosis (also known as Koch's bacillus; a specie of pathogenic bacteria from the family of mycobacteriaceae) that mainly affects lungs. It is derived from a Latin word; tubercle–Round nodule/ swelling & osis-condtions.¹ It is transmitted through air droplets from one person to another. Broadly speaking, it is pulmonary in nature but can be extra-pulmonary (affecting brain, gastrointestinal tract, GIT, kidney, spine, etc). Once the bacteria enter the body, it becomes dormant but becomes active when the person is immune-compromised. Signs and symptoms are bad cough for 3 weeks or longer, chest pain, weight loss, chills, fever, night sweats and bloody sputum. Some-times people get affected but symptoms do not appear, this is called inactive/ latent TB.²

TB is not a new disease rather its history goes beyond centuries. It is the 13th dominant cause of death globally and secondary to the COVID-19 in death rate. Recently in 2021, WHO reported mortality of 06 million males, 3.4 million female adults and 1.2 million children.³

TB has been a long endemic disease in Pakistan. According to WHO, Pakistan has 500,000 active cases per annum. Pakistan has been categorized 5th country among high burden countries worldwide & 4th highest prevalent state of MDR-TB globally. Many types of the specimens have been obtained for the diagnosis of tuberculosis such as sputum, pus and tissue biopsy etc but sputum specimen is the gold standard specimen for pulmonary TB patients.

The fight against TB and to achieve the target of its eradication depends heavily upon the early diagnosis and the adequate treatment of infectious individuals. TB diagnostic tool deficiencies have impeded efforts to combat TB. Smear microscopy, chest X-rays, and TB cultures are among other traditional TB diagnostic techniques, which have rarely proved effective in this regard.⁴ Microscopy only detects 36-43% of TB cases as positive and leaving the remaining cases undiscovered and depriving them from the necessary

treatment. Chest X-rays do not always detect the TB.

An innovation in this field is the availability of GeneXpert since 2010. The Polymerase Chain Reaction (PCR), which recognizes a particular gene and amplifies it to detectable levels, is used to detect TB at the molecular level in GeneXpert. It is superior to previous diagnostic techniques because it also detects rifampicin resistance and provides results in 2 hours.⁵ WHO recommends that next-generation Xpert® MTB/RIF Ultra (Xpert Ultra) cartridge should be used in place of the old GeneXpert cartridge. This cartridge is more accurate for detecting rifampicin resistance. It is also beneficial in detecting bacilli in specimens with low numbers, in the paediatric tuberculosis meningitis, in HIV-infected adults, and in other conditions.⁵

TB is a fatal transmissible disease. A lot of tests are there for its diagnosis in which visual microscopy is considered gold standard despite the fact that it has low sensitivity and takes longer time to give result.⁶ On the contrary, gene expert is more sensitive. In this study we tried to evaluate the sensitivity, specificity, and predictive values by comparing gene xpert and visual microscopy through sputum sample. We retrospectively reviewed sputum sample of 170 patients from January 2015 to November 2015 for gene xpert and visual microscopy and result extracted from this data were 86.8%, 93.1%, 78.5%, 96% by gene xpert for sensitivity, specificity, PPV, NPV respectively.⁷ By visual microscopy, results were 22.2% and 78.5% for sensitivity and specificity respectively.

For TB detection, gene Xpert is highly sensitive and specific, but its accuracy varies according to infectious sites and diagnostic specimen. To determine its accuracy, the researchers opted for meta-analysis. They found that in pulmonary TB gene Xpert gave maximum efficacy when gastric juice and sputum specimens were used as samples. For bone and joint TB, the biopsy and joint fluid specimen was used. However, it could not differentiate among

TB meningitis, TB pleuritis and unclassified TB. However for detection of TB by gene xpert technique, the adequate amount and type of specimen is needed.⁸

For detection purposes, two techniques are used, smear microscopy and gene Xpert. Smear microscopy has low specificity, but it is very cost effective and commonly used techniques.⁹

On the other hand, gene Xpert is highly specified and is recommended by WHO. The researcher collected 175 samples in order to diagnose pulmonary and extra-pulmonary TB. They performed ZN staining and gene xpert. Out of 175 samples, 40 were positive with gene xpert and remaining were positive with microscopy. Distribution of sample was not uniform in this study so the specificity and sensitivity could not be detected.¹⁶

TB is one of the challengers especially in developing countries. According to WHO, it causes around 5000 deaths per day. ZN staining is a famous technique for early diagnosis as acid fast bacilli is seen bright red with blue background in smear of this stain. However, 5000 to 10000 bacilli in sample are required to give positive result. That's why culture is considered gold standard technique, but it takes 4-6 weeks to give result. PCR technique gives effective result as only 2 bacilli per ml are enough in sample to give positive results. This descriptive study was carried out from Dec 2012 to March 2014, in Mayo Hospital Lahore, where 403 patients were asked to submit 2-4 ml of sputum sample. Smear positivity was around 67.5% while gene xpert positivity was around 77.4%. Positive predictive value was same for both procedures. However, specificity, sensitivity and negative predictive value was high for gene xpert.⁴

TB is a primary cause of death after HIV worldwide. Pakistan is rated 5th among 22 high burden countries. More than half cases of TB coexist with HIV. Every year 10% extrapulmonary cases are reported. High prevalence in Pakistan is due to lack of timely and proper diagnosis.⁴

A study was conducted from January 2013 to January 2015 in which gene xpert and LED-FM microscopy were compared. They collected about 737 samples from the suspected individuals. Out of which 53% were male and 47% were female and 13% results were positive by the gene xpert. Sensitivity ranged from 50 to 100%. However, the specificity was 100% while 18% results were positive in culture. Samples which gave positive result by gene xpert technique were also positive in culture technique. Only 7% results were positive by LED-FM microscope technique. Specificity to this test was 100%. So, Gene xpert was considered an effective and early diagnostic tool.¹⁰

Using sputum culture as a reference standard test in as systematic review and meta-analysis, the GeneXpert had a pooled sensitivity of 86% (it detects 86% of true positive patients) and specificity of 98% (it can report 98% of patients without the disease as test negative). Comparatively, smear microscopy had sensitivity of 36-43%.¹¹

In a large randomized clinical trial evaluating the effect of replacing GeneXpert with the smear microscopy on patient and program outcomes in South Africa, the churchyard and the colleagues found that there was no difference between the GeneXpert and microscopy arms in terms of the mortality, proportion starting tuberculosis treatment, and initial loss to follow up. However, they noted a 50% higher rate of people with bacteriological confirmation of the TB in the Gene-Xpert group thereby allowing a reduced time to treatment initiation.¹²

One of the biggest obstacles in the fight against TB is the underutilization of GeneXpert, which leaves many TB patients unidentified, particularly in nations with high TB-HIV burdens like Pakistan. Recent research has demonstrated that the Gene-Xpert diagnostic technology is under-utilized in Pakistan, which has resulted in a low rate of TB case detection.

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Objective

To compare the efficacy of Gene Xpert with visual microscopy of sputum.

Methodology

Conflict of Interest; There is no conflict of interest.

Type of study; Observational retrospective comparative study.

Duration of study; One year. April, 2022 – April, 2023.

Study setting: Pulmonology department of Allama Iqbal Memorial Teaching Hospital, Sialkot Pakistan.

Sample size: One hundred and forty-eight (148) TB suspected patients.

An observational descriptive retrospective comparative study was conducted from April 2022 - April 2023. It was a comparative study on 148 patients. Sputum samples were collected from each patient & on these samples Visual Microscopy & GeneXpert were performed. The data was analysed on SPSS 25. Version of descriptive statistics & results were compared on the basis of positive & negative predictive values.

Type of Techniques

Smear Microscopy

On contrary, **Visual Microscopy of sputum** is most common diagnostic criteria. This is done by using by ZN (Ziehl Neelsen) staining.¹¹

Results

Out of 148 patients only 13 were in age group of 10-18 years, while 28 were in 19-29 years, however 29 were of 30-40 years, 55 were of 41-60 years and 23 patients were in age group of >60 years, respectively.

For the purpose of confirmation of TB, smear microscopy and GeneXpert were performed. Referring to microscopy, 8, 24, 21, 39 and 17 patients were reported positive from age groups of 10-18, 19-29, 30-40, 41-60 and >60, respectively. 5, 4, 8, 16 and 6 patients were reported negative with TB from age groups of 10-18, 19-29, 30-40, 41-60 and >60, respectively, as shown in table 4.1.

All 48 selected samples were reported positive when GeneXpert technique is used. Fig 4.1, 4.2, 4.3 and 4.4 shows the overall comparison of data of different age groups with different diagnostic techniques.

Age Group	Total Patients	Microscopy		GeneXpert	
		+ve	- ve	+ ve	- ve
10-18	13	8	5	13	-
19-29	28	24	4	28	-
30-40	29	21	8	29	-
41-60	55	39	16	55	-
>60	23	17	6	23	-

Table 4.1 shows age group, total number of patients and techniques of identification of MTB.

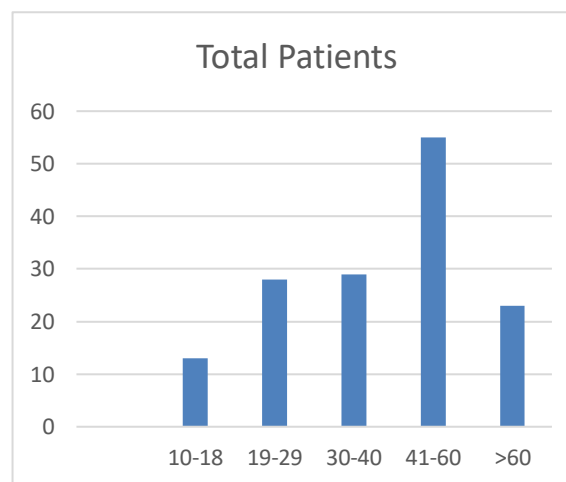
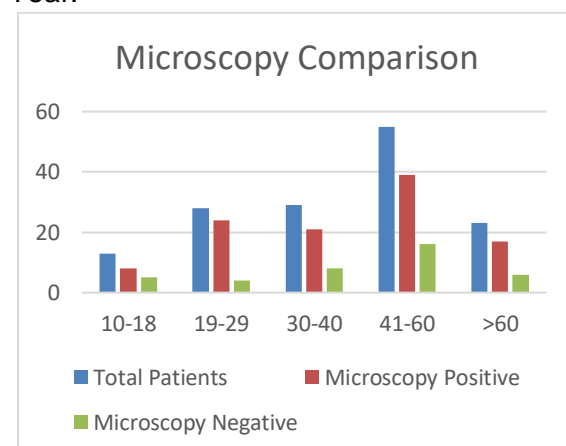


Fig 4.1 showing age group comparison of patients presented at Pulmonology Department of Allama Iqbal Memorial Teaching Hospital, Sialkot, Pakistan in One Year.



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Fig 4.2 showing comparison of total number of patients, indicated by blue bar, patients who were reported positive for TB with reference to microscopy technique with orange bar and patients who were reported negative for TB reference to microscopy technique with grey bar.

One-Way AOV for: Microscopy Results
 One-Way AOV for: V001 V002 V003 V004

Source	DF	SS	MS	F	P
Between	3	1307.73	435.909	3.09	0.0591
Within	15	2116.80	141.120		
Total	18	3424.53			

Grand Mean 20.842 CV 57.00

	Chi-Sq	DF	I
Bartlett's Test of Equal Variances	4.22	3	0.2388
Cochran's Q	0.4210		
Largest Var / Smallest Var	10.422		

Component of variance for between groups 62.2332
 Effective cell size 4.7

Variable	N	Mean	SE
V001	4	25.000	5.9397
V002	5	29.600	5.3126
V003	5	21.800	5.3126
V004	5	7.800	5.3126

Fig 4.3 shows one-way ANOVA analysis of patients' age group and microscopic technique, indicating value of P >0.05, showing no effect of variable on each other.

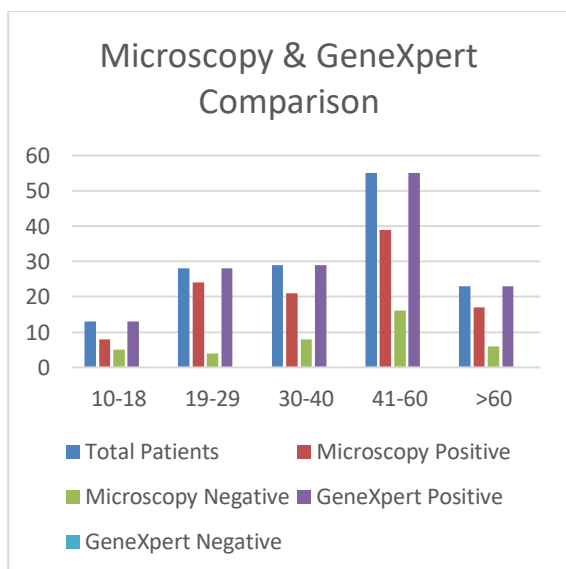


Fig 4.4 showing comparison of total number of patients, indicated by blue bar, patients who were reported positive for TB with reference to microscopy with orange bar, patients who were reported negative for TB reference to microscopy technique with grey bar, patients who were reported negative for

TB reference to GeneXpert technique with yellow bar and patients were reported as negative using GeneXpert with multi-bar diagram.

Discussion

A study conducted in Ethiopia in 2018 shared that different methods with varied sensitivity and specificity for TB diagnosis have been developed. Rapid and specified diagnosis is important for the prevention of Mycobacterium tuberculosis.¹³ Regardless of the multiple methods developed for the diagnosis of MTB, there is difficulty in differentiating between patients with active or latent disease. Physicians still rely on typical methods of diagnosis such as Ziehl Neelsen (ZN) staining, sputum culture, fluorochrome staining, gastric lavage and the other non-typical methods. Despite of the fact that tuberculin test has helped the physicians in the diagnosis of MTB, its interpretation is difficult because sensitization with non-tuberculosis mycobacteria leads to false-positive results.

However, researchers found after a study in Ghanna that methods used for the lab diagnosis of MTB are still progressing to achieve more rapid, less costly & specified results. Ziehl Neelsen (ZN) staining and culture for MTB remained as the primary diagnostic method. Molecular technologies such as GeneXpert, DNA sequencing or PCR can be used for identification of definitive species and allowing for direct detection of MTB without depending upon the culture growth.¹⁴

Microscopic examination of stained smears is known as a rapid and less expensive screening method for MTB. Relating to the gram positive bacteria based on peptide-glycan in cell wall mycobacteria are not reliably detected with the typical Gram staining method. However, in the presence of phenol and heat, carbol fuchsin dye is used during Ziehl–Neelsen staining with methylene blue as counterstain. These cell-wall dye complexes are unaffected to de-staining with mineral acids, mycobacteria are referred as “acid-fast bacilli” or “AFB”, thus Acid-fast staining serves as a rapid and

inexpensive screening method for AFB.¹¹ The results of AFB staining appear within 24 hours of sample collection. The samples had positive results with a high predictive value but have relatively low sensitivity.¹²

Due to the severity of tuberculosis disease and concerns regarding transmission, early diagnosis of active disease is essential. Thus, majority of molecular tests have specifically focused on the identification of MTB. However, with the evolution of molecular biology techniques, they have been advances in nucleic acid based amplification and hybridization, which aid in rectifying the flaws in the diagnosis of MTB.¹⁵

The detection of MTB DNA in specimens by polymerase chain reaction (PCR) is an optimistic approach for the rapid diagnosis of MTB. Gen-Xpert MTB/RIF test is an automated & cartridge based system that uses a closed amplification system that benefits in the reduction of the potential risk of cross-contamination between specimens. It is simple to perform and no advanced machine is required. It also has the benefit of providing the information regarding the rifampin resistance which is also a predictor of MDR TB. Awais and his colleagues conducted a study in Bangladesh and found these results.¹⁶

Although the microscopic evaluation is less expensive and routinely and commonly performed at every health sector level but gene Xpert is more specific & sensitive regarding the diagnosis of the specific specie of MTB & the resistance against rifampin or isoniazid which benefits more in the treatment and prevention of the transmission of MTB disease.^{17, 18}

Our study also coincides with the above said research findings.

Conclusion

GeneXpert has become superior to AFB culture in the diagnosis of TB, due to its high sensitivity, specificity and speed. Now, it is acceptable to make a tuberculosis diagnosis using GeneXpert. However for other extra-pulmonary tuberculosis, the AFB Culture is still considered gold standard technique.

Other available diagnostic techniques are also valuable in this regard.

Disclaimer

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Sample data was collected from Pulmonology department of Allama Iqbal Memorial Teaching Hospital, Sialkot, Punjab, Pakistan by the approval form respective department.

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