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ORIGINAL ARTICLE

Assessment of diagnostic accuracy of Ziehl-Neelsen microscopy and GeneXpert MTB/ RIF assays from pulmonary and extra pulmonary specimens in a tertiary care hospital setting.

Nadia Wali[1], Huma Amin[2], Sara Arif[3], Sadaf Kazmi[4], Fatima tuz Zuhra[5], Sadaf Waris[6]

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ABSTRACT… Objective: To compare the diagnostic accuracy of GeneXpert over ZN microscopy in pulmonary and extra pulmonary suspected tuberculosis samples. **Study Design:** Retrospective Cross-sectional study. **Setting:** Tertiary Care Hospital, Rawalpindi. **Period:** January 2024 to June 2024. **Methods:** All suspected tuberculosis cases from pulmonary and extra pulmonary samples were subjected to ZN microscopy and Gene Xpert MTB/RIF. Samples irrespective of age and gender were included in the study and spot specimens were excluded. Frequency and percentages were calculated for variables using SPSS v.26. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ZN microscopy and GeneXpert were also analyzed. **Results:** Out of total 813 suspected pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (EPTB) specimens, 467(57.4%) were males while 346(42.5%) were females. Among all the samples, 695 (85.48%) were pulmonary and 118 (14.51%) were extra-pulmonary samples. The total number of samples stained positive with ZN were 106 (13.03%) whereas, sample detected postive on GeneXpert MTB/RIF were found to be 124 (15.25%). Out of all ZN postive samples detected by ZN staining method, 103 (97%) were PTB and 3 (03%) were EPTB samples. Whereas the positive samples detected by GeneXpert MTB/RIF comprised of 109 (88%) PTB and 15 (12%) were EPTB samples. Furthermore, sensitivity and specificity of Gene Xpert MTB/RIF was found to be 85.48% and 100% respectively, with a 100% PPV and 97.4% NPV in this study. **Conclusion:** GeneXpert MTB/RIF has a higher sensitivity and specificity compared to conventional ZN smear microscopy. GeneXpert has detected more positive cases from both PTB and EPTB specimens.

Key words: GeneXpert, MDR-TB, Pulmonary Tuberculosis.

INTRODUCTION

Tuberculosis (TB) currently represents a major infectious disease worldwide. In 2019, the World Health Organization (WHO) estimated 1.2 million deaths and 7.1 million new cases (WHO, 2020).[1] Although TB mainly affects the lung parenchyma, Mycobacterium tuberculosis can spread to extra-pulmonary sites. Pakistan has still high TB burden and is ranked among the top five countries globally in TB incidence.[2]

TB accounts for both pulmonary (PTB) and extrapulmonary (EPTB) cases. In 2019, EPTB cases accounted 16% of the 7.5 million incident cases worldwide.[3] EPTB can manifest as either primary EPTB, occurring at the initial infection site, or secondary EPTB, which typically results from hematogenous or lymphatic dissemination. Secondary EPTB can be due to the reactivation of latent TB (LTBI), ingestion of infected sputum, or local spread from adjacent organs.[4] The diagnosis of EPTB is particularly challenging as most cases have very different presentation and ZN microscopy is negative. The incidence of TB has also been on the rise, particularly among individuals suspected of having TB but with negative smear tests, in regions with a high HIV prevalence. In such areas, the emergence of TB mutations leading to drug-resistant strains has become increasingly prevalent. Timely and

1. MBBS, M.Phil, Ph.D (Microbiology), Professor Pathology, Akhter Saeed Medical and Dental College, Lahore.
2. MBBS, M.Phil, Ph.D (Hematology), Associate Professor Pathology, Bahria University Medical and Dental College, Islamabad.
3. MBBS, M.Phil (Microbiology), Lecturer Pathology, Akhter Saeed Medical and Dental College, Lahore.
4. BDS, M.Phil (Oral Pathology), Assistant Professor Pathology, Akhter Saeed Medical and Dental College, Lahore.
5. MBBS, M.Phil, FCPS, Assistant Professor Pathology, Rawalpindi Medical University, Rawalpindi.
6. BDS, M.Phil (Oral Pathology), Associate Professor Pathology, Akhtar Saeed Medical and Dental College, Lahore

Correspondence Address:
Dr. Sara Arif
Akhter Saeed Medical and Dental College, Lahore.
dr.saraarif@gmail.com

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accurate TB diagnosis plays a pivotal role in the implementation of effective TB control strategies, facilitating the early commencement of treatment of patients and those afflicted with multidrug-resistant Tuberculosis (MDR-TB).[5]

Worldwide, around 600 children presented with TB every day. Among the estimated 1 million children who developed active TB in 2019, approximately 70% went undetected or were misdiagnosed by healthcare providers (WHO 2020)[6], as ZN smear microscopy is usually negative in paediatric patients. The acid-fast bacilli (AFB) smear is a commonly employed and cost-effective diagnostic method for pulmonary tuberculosis. Despite its widespread use, it exhibits limited sensitivity and requires a bacterial concentration of 10,000 colony forming units/mL to yield a positive result when examined under a microscope. Consequently, samples with low bacterial counts may yield negative reports.[7] In the year 2010, the WHO recommended the utilization of the GeneXpert MTB/RIF. This automated, cartridge-based molecular test was endorsed as the primary diagnostic tool, with the aim to identify M. tuberculosis detection rates and enhance the diagnosis of rifampicin (RIF) resistance in cases of pulmonary and EPTB specimens.[8] GeneXpert MTB/RIF detection limit is 131 Colony Forming Unit (CFU) per ml which is more sensitive than AFB.[9] Alternatively, a novel, rapid, automated nucleic acid amplification test (NAAT) was also recommended for the initial diagnosis of patients suspected of having pulmonary multidrug-resistant TB or HIV-associated pulmonary TB. This test offers the capability to simultaneously identify TB by detecting Mycobacterium tuberculosis DNA and also detects a majority of the mutations associated with rifampicin resistance, a strong indicator of MDR-TB.[10] Annually, approximately 3.3 % of new TB patients and approximately 20 % of previously treated patients become infected with MDR-TB, leading to higher mortality rates.[11]
In a significant development in late 2013, the WHO expanded its recommendations to encompass the diagnosis of TB in children as well as specific forms of EPTB.[12] Mycobacterial culture is usually not recommended as an initial diagnostic investigation. The conventional solid culture method (Lowenstein Jensen medium) has a better sensitivity as its detection limit is 10-100/ml of specimen[13], but it takes around 6-8 weeks to get the results. Other liquid culture methods such as BACTEC or Mycobacterium Growth Indicator Tube (MGIT) offer faster results compared to other techniques. However, their implementation is limited by high costs and logistical challenges, making it impractical to deploy them at district microscopy centers or in remote areas where resources are limited.[14]

As TB is highly prevalent in Pakistan and is also communicable and infectious disease, hence it is crucial for healthcare workers to timely and accurately diagnose and manage tuberculosis to avoid its spread. Moreover, diagnostic test like GeneXpert is also able to identify rifampicin resistance which helps in timely initiation of therapy. Owing to the benefits of the GeneXpert MTB/RIF test, the present study was conducted to compare the diagnostic efficacy of GeneXpert MTB/RIF assay with Ziehl Neelsen staining (smear & microscopy) for the diagnosis of PTB and EPTB specimens in a tertiary care hospital.

METHODS

This retrospective cross-sectional study was carried out at Rawalpindi District Headquarter Hospital. All ethical requirements were duly addressed before the start of this study and approval was obtained from IRB under letter no 1370/RTH,Rwp. This study involved participation of 813 patients aged one year and older. All samples were subjected to ZN smear microscopy and GeneXpert MTB/RIF testing. The data collection period spanned from January 2024 to June 2024. The study included both pulmonary and extra-pulmonary samples, including primary cases of Tuberculosis and early morning specimens. The study excluded only spot specimens. All other pulmonary and extra-pulmonary tuberculosis samples were included.

Non-probability consecutive sampling was done and data collection included demographic information of patients, type of samples and cases from different wards. Non-sterile clinical samples like sputum and pus were pre-treated according

to the conventional N-acetyl-L-cystine-NaOH decontamination procedure. Specimens from sterile sites were centrifuged and used directly for further procedure. Each sample was subjected to ZN smear microscopy and GeneXpert MTB/RIF test results for analysis. A pulmonary and extra-pulmonary TB case was identified as a patient with positive ZN smear or Gene Xpert MTB/RIF test result. The effectiveness of ZN smear microscopy, as a diagnostic test was assessed using Gene XpertMTB/RIF as the standard investigation.

The smears were arranged in a sequential manner on a staining bridge, with the smear side facing upward, and then soaked with 0.1% Carbol Fuchsin. Afterward, the smears were subjected to heat and allowed to stay for few minutes, removed just before the start of boiling. After that slides were thoroughly rinsed with water and left to drain. Decolourization was carried out using a 25% sulphuric acid solution for 5 minutes, followed by thorough rinsing and drainage. Later, the smears were counterstained with a 0.1% methylene blue solution for 1 minute, followed by rinsing with water. Finally, the smears were air-dried and examined under the microscope, using the oil immersion (100X) objective for detailed analysis.

The Xpert MTB/RIF assay was carried out following the guidelines provided by the manufacturer (Cepheid Inc., Sunnyvale, CA, USA). Samples were collected and placed in the provided containers, then treated with a sample reagent at a ratio of 2:1. These treated samples were then incubated for 15 minutes at room temperature. After this incubation period, precisely 2 milliliters (2 ml) of the reagent-treated sample were transferred into the sample chamber of the Xpert cartridge. The Xpert cartridge was then inserted into the GeneXpert instrument system and started. Following a processing time of 90 minutes, the results were generated.

Statistical Analysis

The data was entered and analysed using the SPSS (version 26.0, USA). Frequency and percentage were reported for variables such as gender, ZN staining, PTB and EPTB samples, Gene Xpert result and Rifampicin resistance.

For comparative analysis between methods, a 2 x 2 table was made for ZN microscopy and GeneXpert MTB/RIF for four distinct categories: true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). The calculation of metrics involved sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).
Sensitivity: TP/ (TP+FN) x 100
Specificity: TN/ (TN+FP) x 100
PPV: TP/ (TP+FP) x 100
NPV: TN/ (FN+TN) x 100

RESULTS

A total of 813 suspected PTB and EPTB specimens were analyzed. The minimum age of patients was one year while maximum was 75 years. The mean age of participants was found to be 35±19.34. Males were found to be 57.4% while 42.5% were females (Table-I).

Out of 813 suspected TB cases, 695 (85.5%) were PTB while 118 (14.4%) were EPTB samples. 126(15.5%) sputum samples,79(9.7%) gastric aspirates, 19(2.3%) fluids and 1(0.1%) CSF, were found to be TB positive cases (Table-II).

The majority of the cases were reported from Rawalpindi 737 (90.7%) followed by cases reported from Islamabad 23 (2.8%) and Azad Kashmir 14 (1.7%) (Figure-1).

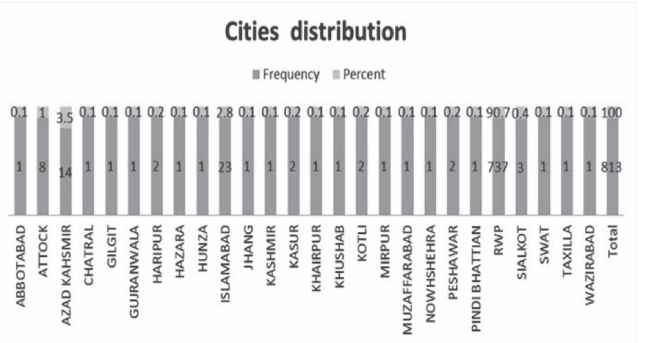

Figure-1. Distribution of suspected cases according to the location

Most of the cases were reported by OPD (74%) followed by TB ward (11%) and Paeds ward (8%) (Figure-2)

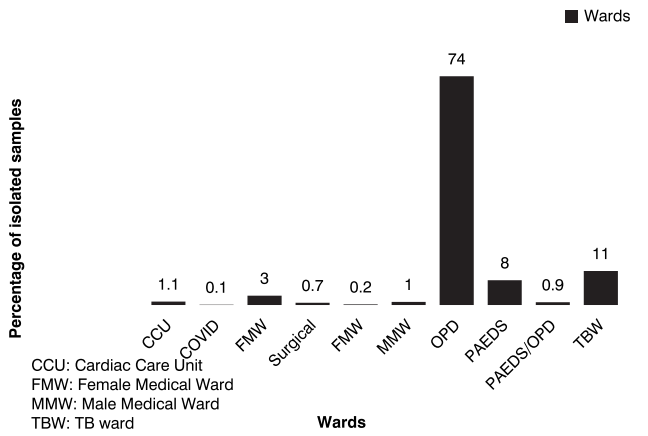

Figure-2. Details of samples from different wards

Smear examination (ZN staining) was positive in 106 (13%) patients. When GeneXpert assay was performed on same samples, 124 (15.3%) samples showed positive for TB. RIF (Rifampicin) resistance was detected in 10 (1.2%) samples and one sample result was indeterminant as shown in Table-III.

Table-IV demonstrates the comparison of test results of two methods under study. Positive cases detected by ZN staining were 106, whereas 707 were found to be negative. While GeneXpert detected 124 positive cases and 689 were found to be negative.

Type of Specimens	Gender		Age Groups					
	Male	Female	1 -18 Years	19-30 Years	31-40 Years	41-50 Years	51-60 Years	≥61 Years
PTB	403	292	138	159	111	91	69	58
EPTB	64	54	35	56	26	16	22	32
Total	467 (57.4%)	346 (42.5%)	173 (21.3%)	215 (26.4%)	137 (16.8%)	107 (13.2%)	91 (11.2%)	90 (11.1%)

Table-I. Gender and age distribution of EPTB and PTB cases

Samples	n (%)
Pulmonary samples	
Sputum	126 (15.5%)
Extra pulmonary samples	
Gastric aspirates	79 (9.7%)
Fluids (pleural fluid, ascetic fluid)	19 (2.3%)
CSF	1 (0.1%)
Pus	1 (0.1%)
Total	226 (27.7%)

Table-II. Frequency of positive pulmonary and extra pulmonary samples

ZN Staining	Detected	Not Detected	Total	
	106 (13%)	707 (87%)	813 (100%)	
Detected PTB EPTB	103 (97.2%) 03 (2.8%)			
M. Tb GeneXpert	Detected	Not detected	Error*	Invalid**
	124 (15.3%)	669 (82.3%)	19 (2.3%)	1 (0.1%)
Detected PTB EPTB	124 (87.9%) 15 (12.1%)			
M. Tb quantitative	High	Medium	Low	Trace
	60 (48.4%)	24.9 (20.2%)	36 (19.3%)	15 (12.1%)
Rifampicin Resistance	Detected	Not Detected	Indeterminant***	
	10 (1.2%)	148 (18.2%)	1 (0.1%)	

Table-III. Frequency of ZN staining, GeneXpert and RIF resistance

*Technical errors with codes
**Unable to interpret the result of sample
*** Defined as the absence of corresponding mutation bands for Rif gene

ZN Staining	GeneXpert		Total
	Positive	Negative	
Positive	106 (TP)	0 (FP)	106
Negative	18 (FN)	689 (TN)	707
Total	124	689	813

Table-IV. Comparison of diagnostic performance of two methods

Sensitivity: 106/ (106+18) x 100=85.4%
Specificity: 689/ (689+0) x 100=100%
PPV: 106/ (106+0) x 100=100%
NPV: 689 (18+689) x 100=97.4%

DISCUSSION

Tuberculosis is a public health threat with an increasing death rate especially in developing countries. Early detection and starting of proper treatment regimen are ultimately important to reduce the mortality rate (World Health Organization 2017). Global incidence rate has increased by 4.6% (new cases from 100000 population per year) from 2020 to 2023.[15] This increase may be due to better diagnostic techniques available like Gene Xpert.

The demographic analysis indicated that tuberculosis predominantly affected younger adults, with a declining trend observed in older age groups. Furthermore, in this study, 57.4% Tuberculosis patients were males while 42.5% were females. Globally, the distribution of TB varies with age and gender. WHO (2023) documented variations in age and gender distribution across different continents where Africa showed a higher prevalence among younger age groups, Asia showed a more uniform distribution across all age brackets. A comprehensive study by Smith et al. (2023) recorded a higher prevalence of tuberculosis among males aged 25-44 years.[16]

Our findings are in concordance with few local studies. In a study conducted in PIMS Hospital, Islamabad higher incidence of detected TB cases (48%) was found in the same age bracket of 29-30 years.[17] A study by Ali et al. (2020) reported a higher incidence of tuberculosis among males aged 15-34 and 55-65 years.[18] However, another research documents a very characteristic trend in different provinces of Pakistan indicating a higher TB prevalence in females in the western provinces as compared to the eastern provinces. This disparity in distribution among different populations may be due to socio-economic differences, variations in healthcare, population densities, and difference in diagnostic modalities, health care accessibility and urbanization of different areas.[19]

Out of 144 positive cases of TB in the present study, 126(87.5%) cases were diagnosed using the pulmonary samples and 18(12.5%) were diagnosed using extra-pulmonary samples. A study also emphasizes upon the importance of considering extrapulmonary samples like gastric aspirates where the clinical presentation is atypical.[20] The geographic distribution of TB cases in Pakistan reveals highest incidence in the province of Sindh followed by Khyber Pakhtunkhwa and Balochistan. The prevalence was higher in rural areas as compared to urban probably owing to limited health facilities, poverty lack of awareness regarding TB in these regions.[21]

Several studies from various regions of Pakistan have different results regarding RIF resistance. A high RIF resistance rate was detected by a study conducted in Narowal (7.1%) which is contradictory to our results i-e 1.2%.[22]

GeneXpert sensitivity result of 85.4% in our study aligns closely with the international meta-analysis done in 2021 that recorded a collective sensitivity of 91% (23). One research conducted in Pakistan revealed 99% sensitivity, highlighting the importance of GeneXpert MTB/RIF in detecting tuberculosis both internationally and locally.[24]

Specificity of GeneXpert recorded in this study is 100% which is in alignment with a few studies which reported the same specificity of 100% regarding Gene Xpert.[25] Similarly, a study conducted in Pakistan documented the specificity of 100% in the KPK population.[26] On the contrary, results

of another research conducted in Bangladesh reported a significantly lower specificity of 88.3% showing that the variation in population can affect the range of specificity of Gene Xpert.[27]

Positive predictive value (PPV) and Negative predictive value (NPV) for Gene Xpert test in our study were documented as 100% and 97.4% respectively. Solanki and Karthek, both have reported a 100% PPV in their studies supporting our findings, whereas the NPV assessed by both had a contrasting difference of 93.88% and 56.5%.[28] NPV assessed by research conducted in Rawalpindi, Pakistan was calculated to be 99% underscoring the utility of Gene Xpert in correctly identifying the true positive cases of TB.[29]

CONCLUSION

The study concludes that GeneXpert MTB/RIF has higher sensitivity and specificity to conventional ZN smear microscopy. Moreover, GeneXpert has identified additional positive cases from both PTB and EPTB samples.

LIMITATIONS

The present study is a single center study, has limited sample size and was carried out over a limited time period.

FUTURE OUTCOMES

The detection of rifampicin resistance can help in the selection of appropriate treatment regimen and help prevent the spread of MDR-TB. Due to the simplicity, sensitivity and specificity of GeneXpert proves to be highly impressive tool for diagnosis of Mycobacterium tuberculosis and rifampicin resistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Nadia Wali:** Design of work, interpretation of data, final revision of article.
2	**Huma Amin:** Data analysis.
3	**Sara Arif:** Drafting, agreement to be accountable for all aspects of work.
4	**Sadaf Kazmi:** Drafting.
5	**Fatima tuz Zahra:** Analysis of interpretation.
6	**Sadaf Waris:** Revision, final approval.