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Diagnostic Utility of GeneXpert MTB/RIF in Extra-Pulmonary Tuberculosis: A Retrospective Study from a Tertiary Care Centre in Western India

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ABSTRACT

Extra pulmonary tuberculosis accounts for a sizeable fraction of tuberculosis cases in India. It is an enigmatic disease with a protean presentation and low bacillary load, posing a challenge to timely diagnosis. Rapid diagnosis is of prime importance in initiating early treatment and halting the spread of drug-resistant organisms. GeneXpert MTB/RIF assay, or CBNAAT, simultaneously detects Mycobacterium tuberculosis and its resistance to rifampicin in a variety of extrapulmonary specimens.

Objective: To assess the diagnostic utility of GeneXpert MTB/RIF in suspected EPTB and specimen-wise positivity with rifampicin resistance patterns.

Materials and Methods: This is a retrospective observational study conducted among a tertiary care hospital in Rajkot, Gujarat, from September 2022 to September 2024. A total of 434 extrapulmonary specimens were lymph node aspirates, pus, pleural fluid, cerebrospinal fluid, ascitic fluid, and synovial fluid examined by GeneXpert MTB/RIF. The demographic and clinical data along with laboratory data were retrieved from the records. Descriptive statistics and chi-square testing were conducted to evaluate the association of MTB positivity with HIV status and sample type.

Results: MTB was positive in 92 out of 434 samples (21.2%). The highest rate of positivity was seen in pus/cold abscess and lymph node aspirations, and the lowest was seen in serous fluids. The rate of MTB positivity was slightly higher among HIV-positive patients than among HIV-negative patients, but the difference was not statistically significant ($p = 0.20$). There was a statistically significant relationship between the type of specimens and the rate of MTB positivity ($p < 0.001$). Resistance to rifampicin was seen in 12 out of 92 samples of MTB-positive specimens, mostly from the lymph node and cerebrospinal fluids.

Conclusion: GeneXpert MTB/RIF is a fast and accurate diagnostic test with varying sensitivity for EPTB diagnosis according to the type of specimen being tested. The resistance results for rifampicin support early initiation of effective therapy and TB control activities within high-prevalent countries.

Keywords: *Extra-pulmonary tuberculosis; GeneXpert MTB/RIF; molecular diagnosis; rifampicin resistance; diagnostic yield.*

INTRODUCTION

Tuberculosis (TB) remains one of the most important infectious causes of morbidity and mortality, with an estimated 10.6 cases of TB disease newly diagnosed annually in the year 2023 worldwide[1]. India alone accounts for almost a quarter of all TB cases globally, making it a high priority for public health to control and manage[2]. Extrapulmonary tuberculosis (EPTB), a form of TB that affects any organ beyond the pulmonary parenchyma, accounts for approximately

20-25% of all cases of TB and has its special diagnostic pitfalls[3]. The incidence of EPTB is even higher in immunocompromises like people living with HIV/AIDS[4].

EPTB is usually diagnosed late because of its nonspecific clinical presentation, deep-seated anatomical involvement, and the low bacillary load in the extrapulmonary specimens[5]. Traditional diagnostic methods like smear microscopy show poor sensitivity in EPTB due to its paucibacillary nature, while culture techniques, which are more sensitive, are cumbersome and resource-intensive[6]. Histopathology and cytology are helpful yet lack microbiological confirmation and cannot establish any information on drug resistance[7]. Such deficiencies commonly lead to an empirical beginning of anti-tubercular therapy, which also leads to overtreatment, delayed detection of drug resistance, and enhanced health-care burden[8].

Nucleic Acid Amplification Tests (NAATs) have introduced a major change in the TB diagnosis landscape. GeneXpert MTB/RIF (cartridge-based Nucleic Acid Amplification Test; CBNAAT) is a completely automated molecular diagnostic technique that can detect M. tuberculosis DNA and RIF-resistance in two hours[9]. Resistance to RIF is a valid marker for MDR-TB, thereby allowing timely commencement of the respective therapy regimen[10]. Due to its high specificity, absence of biosafety concerns, and short turn-around time, the World Health Organization recommends GeneXpert MTB/RIF as a first-line tool for the diagnosis of both pulmonary and Extra pulmonary TB[11].

There have been numerous systematic reviews and meta-analyses showing better diagnostic performance of GeneXpert MTB/RIF compared with smear microscopy in patients with extra pulmonary TB cases involving lymph nodes, cerebrospinal fluid, and pus specimens[12][14]. Despite the guidelines, it has been observed that there have been very few studies assessing the existing real-world performance evaluation of GeneXpert MTB/RIF in extrapulmonary specimens in high TB prevalence countries including west India.

Objectives

1. To evaluate the diagnostic yield of the GeneXpert MTB/RIF test on suspected extra-pulmonary tuberculosis patients.
2. To determine the distribution of MTB positivity among various types of extrapulmonary samples.
3. To find out the prevalence of rifampicin resistance detected by GeneXpert in EPTB.

MATERIALS AND METHODS

Design and Setting:

It was a retrospective observational study, which was performed in the Department of Respiratory Medicine, PDU Government Medical College & Civil Hospital, Rajkot, Gujarat, India. This is a tertiary level referral hospital in Rajkot, serving both urban as well as rural areas of western Gujarat.

Study Duration

The research was conducted over a two-year period ranging from September 2022 to September 2024.

Study Population

All patients suspected of having EPTB, in whom the extra-pulmonary sample had been sent for GeneXpert MTB/RIF (CBNAAT) test, were included in the study. These patients were suspected to be suffering from EPTB depending on the judgment of the attending physician in the form of clinical features, radiological findings, and corresponding lab work.

Sample Size

A cumulative total of 434 extrapulmonary samples satisfying the inclusion and exclusion criteria were evaluated during the conduct of this study.

Inclusion criteria:

- Extrapulmonary samples from patients of any age group and both sexes
- Samples included lymph node aspirations or biopsies, pleural fluid, pus (cold abscess), cerebrospinal fluid (CSF), ascitic fluid, or synovial fluid

Exclusion Criteria

- Sputum, blood, and urine specimens
- Extrapulmonary specimens with blood contamination
- Inadequate or improperly collected specimens

Sample Collection and Processing

All the extrapulmonary samples were processed in aseptic conditions, handled in sterile falcon tubes, with around 5 mL from each sample being processed for each case. The samples were mixed with the reagent sample buffer provided by the manufacturer in a prescribed ratio, then processed with incubation for liquefaction and inactivation of Mycobacterium.
The processed samples were then loaded into GeneXpert MTB/RIF cartridge kits, which were subsequently analyzed using the GeneXpert MTB/RIF machine (Cepheid, Sunnyvale, CA, USA), as per the guidelines of the National Tuberculosis Elimination Program.

GeneXpert MTB/RIF
The GeneXpert MTB/RIF test simultaneously identifies genomic DNA from the Mycobacterium tuberculosis complex, as well as resistance to rifampicin, through the rpoB gene region. The results were automatically interpreted, providing output in the form of:

- MTB detected / MTB not detected
- Invalid or error results

Data Collection
The demographic as well as clinical details, such as the ages, gender, HIV status, co-existing conditions, as well as prior experience of antitubercular treatment, were collected retrospectively on a pre-tested structured proforma from lab registers.

Pilot Testing
Before the full data was extracted, a pilot test of 10 cases was done to check its feasibility, the understandability of the data variables, and the consistency of data gathering. Changes were implemented prior to the compilation of data.

Ethical Considerations
The research protocol was approved and cleared by the Institutional Ethics Committee. Since it involved a record-based study, the risks to participants were low. This study maintained patient confidentiality and collected data anonymously. The method for obtaining informed consent followed institution procedures.

Statistical Analysis
The data collected was entered into Microsoft Excel and then subjected to analysis based on descriptive statistics. Categorical data was represented in terms of frequency and percentage, whereas quantitative data was represented in terms of mean scores.

RESULTS
A total of 434 clinically suspected extra-pulmonary tuberculosis (EPTB) patients were included in the study. Descriptive and inferential statistical analyses were performed to assess associations between key variables and GeneXpert MTB/RIF positivity.

Table 1. Socio-demographic characteristics of study participants (N = 434)

Variable	Category	Number (%)
Sex	Male	303 (69.8)
	Female	131 (30.2)
Age group (years)	<20	42 (9.7)
	20–29	78 (18.0)
	30–39	96 (22.1)
	40–49	113 (26.1)
	≥50	105 (24.2)
Mean age (years)	—	39
HIV status	Positive	61 (14.1)
	Negative	373 (85.9)

The study population demonstrated a male predominance (male: female = 2.3:1). Most patients were aged 30–49 years, indicating higher EPTB occurrence in the economically productive age group. HIV co-infection was present in 14.1% of patients.

Table 2. Overall GeneXpert MTB/RIF results in extrapulmonary samples

Result	Number	%
MTB detected	92	21.2
MTB not detected	342	78.8

Total	**434**	**100**

GeneXpert MTB/RIF confirmed MTB in **21.2%** of extrapulmonary specimens. The majority of samples were MTB-negative, reflecting the known diagnostic difficulty in paucibacillary EPTB.

Table 3. Association between HIV status and MTB detection by GeneXpert MTB/RIF

HIV status	MTB detected n (%)	MTB not detected n (%)	Total	χ^2	p-value
HIV positive	17 (27.9)	44 (72.1)	61		
HIV negative	75 (20.1)	298 (79.9)	373	**1.61**	**0.20**
Total	**92**	**342**	**434**		

MTB positivity was higher among HIV-positive patients (27.9%) compared to HIV-negative patients (20.1%). However, this difference was not statistically significant ($\chi^2 = 1.61$, $p = 0.20$).

Table 4. Sample-wise distribution and MTB positivity by GeneXpert MTB/RIF

Sample type	Total samples	MTB positive n (%)
Lymph node aspirate (FNAC)	133	40 (30.1)
Pleural fluid	127	17 (13.4)
Pus / cold abscess	45	26 (57.8)
CSF	67	5 (7.5)
Ascitic fluid	52	2 (3.8)
Synovial fluid	10	2 (20.0)
Total	**434**	**92 (21.2)**

Significant variability in MTB detection was observed across different extrapulmonary specimens. Pus/cold abscess and lymph node aspirates showed higher positivity, whereas pleural fluid and CSF demonstrated lower yields.

Table 5. Association between sample type and MTB positivity

Sample category	MTB detected	MTB not detected	Total	χ^2	p-value
Lymph node	40	93	133		
Pleural fluid	17	110	127		
Other samples*	35	139	174	**32.6**	**<0.001**
Total	**92**	**342**	**434**		

*Other samples include pus, CSF, ascitic, and synovial fluids.

There was a statistically significant association between the type of extrapulmonary specimen and MTB detection ($\chi^2 = 32.6$, $p < 0.001$), indicating that diagnostic yield of GeneXpert MTB/RIF varies significantly by specimen type.

Table 6. Rifampicin resistance among MTB-positive extrapulmonary samples (n = 92)

Sample type	MTB positive	Rifampicin resistant n (%)
Lymph node aspirate	40	6 (15.0)
CSF	5	3 (60.0)
Pleural fluid	17	2 (11.8)
Pus / cold abscess	26	1 (3.8)
Ascitic & synovial fluid	4	0 (0.0)
Total	**92**	**12 (13.0)**

Rifampicin resistance was detected in 13.0% of MTB-positive cases. Due to the small number of resistant cases, statistical testing for association between sample type and rifampicin resistance was not performed**,** as cell counts were insufficient for reliable inference.

DISCUSSION

Extra-pulmonary tuberculosis (EPTB) is a considerable challenge in terms of diagnosis due to its nonspecific presentation and low bacterial load, which is particularly true in high disease burden countries like India[1]. There has been a high risk of increased morbidity, inadequate empirical therapy, and the potential oversight of resistant cases among patients with EPTB due to prolonged diagnosis. This study emphasizes the utility of GeneXpert MTB/RIF in speedy microbial confirmation of EPTB and the identification of resistance to rifampicin.

In the current study, GeneXpert MTB/RIF sensitivity for M. tuberculosis detection in clinically suspected cases of EPTB turned out to be 21.2%, which is well comparable to the existing literature reports for both Indian and international studies[5,14,15]. This mild sensitivity could be well attributed to the fact that the detection of EPTB has some limitations due to low sensitivity by smear microscopy and longer culture times[6]. Thus, the current work further reinforces that GeneXpert could be an ideal diagnostic aid for rapid detection.

Specimens-wise, there was a considerably higher detection rate of MTB in lymph node aspirates and pus compared to serous fluids. This is also in agreement with what is present in the literature, as pus and lymph node fluid usually have a heavier bacillary load compared to pleural, ascitic fluid, or cerebrospinal fluid[16,17]. While GeneXpert is less sensitive in serous fluids owing to low bacillary loads, its turnaround time is still useful, particularly in cases like tuberculous meningitis, where a quick diagnosis can be a matter of life-and-death[18,19].

Another critical observation of this study was the identification of resistance to rifampicin in 13.0% of MTB-positives in EPTB. Prompt identification of rifampicin resistance, a marker of multidrug-resistant tuberculosis, is a key component of providing appropriate treatment strategies.[20] The identification of drug resistance in EPT samples confirms the need for drug susceptibility testing in all samples, according to the WHO, with GeneXpert MTB/RIF as a prime tool in all forms of tuberculosis[21]. This is especially true in high-burden settings, including resource-poor countries, with a high potential of transmitted resistance, as in this scenario.

CONCLUSION

GeneXpert MTB/RIF is an important tool that has helped in the fast and accurate diagnosis of both the confirmation of extrapulmonary tuberculosis and the detection of rifampicin resistance. Its sensitivity depends on the type of sample used, being higher in the case of aspirates from the lymph nodes, pus, versus serous fluids. Rapid molecular diagnosis has also helped in the early use of appropriate therapy. Improvement in the use of the GeneXpert tool would play an important role in the proper management of extrapulmonary tuberculosis.

Recommendation

- GeneXpert MTB/RIF should be applied as the initial testing procedure for possible cases of extrapulmonary TB for the rapid diagnosis and early detection of rifampin resistance.
- Specimen-specific methods for diagnosis must be emphasized with the prime focus on lymph node aspirates and pus.
- UC-DRT and TB/HIV service delivery should be improved to enable appropriate and timely treatment as a measure towards TB control.

LIMITATIONS

This particular study has some limitations. There could have been some incompleteness in clinical data and possible impairment in sample quality due to the study's retrospective nature. Culture and histopathology were not employed as reference standards, thereby making it impossible to calculate the sensitivity and specificity. There could also have been restrictions in performing the statistical analysis regarding resistance due to a small number of rifampicin-resistant samples. Nonetheless, the study holds important results on the use of GeneXPT in EPTB.

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Conflicts of interests: The authors declare no conflicts of interest.
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