



Original Article

Role of GeneXpert MTB/RIF assay for the diagnosis of cervical lymph node tuberculosis and rifampicin resistance

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ABSTRACT

Objectives: Tuberculosis (TB) of lymph node (TB lymphadenitis) is one of the most common forms of extrapulmonary TB (EPTB) whose diagnosis is critically challenging. Although new diagnostic methods have been developed, especially in patients without a history of TB, the cervical tuberculous lymphadenitis diagnosis is still elusive. This study assessed the applicability of GeneXpert in early diagnosis of EPTB, especially cervical lymphadenopathy. **Materials and Methods:** The study was conducted in a tertiary care hospital from January 2018 to December 2020 at the department of microbiology. All the samples of cervical lymph node tissue and lymph node aspirate were followed as per the routine protocol for mycobacterial identification. The sample was divided into two parts: one was used for the new molecular-based GeneXpert MTB/RIF assay and the second one was tested by direct and concentrated acid-fast bacilli microscopy by Z-N staining and culture for the detection of MTB. **Results:** Among the 145 samples tested, the GeneXpert detected the DNA of MTB in 89 samples (61.37%), whereas the culture test was positive in 42 (28.93%) specimens. GeneXpert also detected 7 rifampicin resistance cases. GeneXpert sensitivity and specificity results were assessed according to culture results. The sensitivity and specificity of the GeneXpert assay were 85.71% and 48.54%, respectively. **Conclusion:** GeneXpert MTB/RIF should be used in conjunction with clinical presentation and other molecular investigation in nonrespiratory specimens.

KEYWORDS: *Extrapulmonary tuberculosis, GeneXpert, Lymph node, Rifampicin resistance*

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INTRODUCTION

Tuberculosis (TB) is the most prevalent and severe infectious disease and major cause of morbidity and mortality throughout the world, with an approximately 10 million new cases occurring annually. India has the highest TB burden in the world and about two-fifth of incident TB cases occur in India. The term extrapulmonary TB (EPTB) has been used to describe the isolated occurrence of TB at body sites other than the lung. The extrapulmonary sites commonly involved are lymph node, pleura, genitourinary tract, bones and joints, meninges, peritoneum, kidney, spines, and growing ends of the bones [1]. TB lymphadenitis (TBL) is one of the most common forms of EPTB whose diagnosis still faces many challenges. In developing countries like India, EPTB accounts for approximately 15%–20% of all cases of TB, and tuberculous lymphadenitis is encountered in nearly 35% of these cases [2]. In high prevalence areas, its incidence is second to that of TB pleuritis and the most common form is mycobacterial cervical lymphadenitis [3].

Conventional ZN smear microscopy and cytology have been used as primary diagnostic tools for TBL in resource-poor settings [4]. However, microscopy lacks sensitivity due to the paucibacillary nature of fine-needle aspirates (FNA). Similarly, mycobacterial culture and drug susceptibility testing are laborious and delayed. Thus, the need for a new rapid and reliable method arose [5].

Several nucleic acid amplification technologies have been developed to diagnose TB rapidly. In 2014, the WHO has recommended GeneXpert over the conventional tests for testing specific nonrespiratory samples such as lymph nodes from patients suspected of having EPTB [6]. This GeneXpert

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MTB/RIF (Xpert) assay is based on nested real-time polymerase chain reaction (PCR) and molecular beacon technology.

In the current study, we evaluated the performance of CBNAAT for the early diagnosis of cervical tubercular lymphadenopathy.

MATERIALS AND METHODS

This was a cross-sectional study conducted in a tertiary care hospital from January 2018 to December 2020 at the department of microbiology. All the samples of cervical lymph node tissue and lymph node aspirate from the suspected patients of cervical lymph node TB and requested for CBNAAT testing were included in the study. Related sociodemographic details and clinical findings of the patient were also documented. Ethical approval was waived for this study.

Ethical committee approval

The study was ethically approved by the institutional ethical subcommittee of medical college by letter Ref No. I.E.S.C./270/21.

Informed consent

Patients' informed consent was taken for the study before the sample process for GeneXpert and traditional methods.

Microbiological investigation

All the samples were followed as per the routine protocol for mycobacterial identification. The gross appearance of the specimen was recorded and categorized as caseous, purulent, and blood stained at the time of specimen collection. The sample was divided into two parts (lymph node tissue was crushed in a sterile mortar): one was used for the new molecular-based GeneXpert MTB/RIF assay and the second one was tested by direct and concentrated acid-fast bacilli (AFB) microscopy by Z-N staining and culture [7,8].

Direct microscopy of smear

Smears were prepared from samples by standard Ziehl-Neelsen staining. All the AFB-positive smears were graded based on the IUATLD scale.

Culture on solid Lowenstein-Jensen media as well as liquid culture in automated MB BacT culture

All the specimens were decontaminated using NALC-NaOH method. The decontaminated specimen was used for the MTB culture. Solid and liquid culture was done as per the standard protocol as well as the manufacturer's instructions. Bottles were placed inside the BacTAlert 3D instrument and incubated at 37°C for 6 weeks. Any bottle which displayed as positive was taken out of the instrument. Similarly, all the samples were also inoculated on Lowenstein-Jensen (LJ) medium and incubated at 37°C for 8 weeks. LJ media bottles were examined for growth every week and LJ. Bottles failing to show any growth after 8 weeks were discarded as negative. Any growths obtained in the bottle were stained by Z-N staining for the detection of AFB. All liquid culture bottles were removed after negative signal at the completion of 6 weeks incubation. Negative signal results were confirmed after Z-N staining.

GeneXpert

The GeneXpert assay is a semiquantitative nested real-time PCR for the detection of MTB and rifampicin resistance. The GeneXpert MTB/RIF (Cepheid) test was performed according to the manufacturer's instructions. Briefly, Xpert sample reagent was added to the sample in 2:1 ratio into a 50 mL centrifuge tube and incubated at room temperature for 15 min. During the incubation period, the samples were mixed by inverting the tubes gently two times every 5 min. Then, 2.0 mL of liquefied sample was transferred to Xpert Cartridge and loaded into GeneXpert Machine, within 2-h Xpert machine provided the result [9].

Statistical analysis

Data were entered in the Excel and analyzed using SPSS v23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) and summarized using mean and standard deviation. Appropriate tests of statistical significance such as Chi-square and paired *t*-test were used. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), test accuracy, and likelihood ratio were calculated by EpiInfo v7.2.5.0 (Dean AG, Arner TG, Sunki GG, Friedman R, Lantinga M, Sangam S, Zubieta JC, Sullivan KM, Brendel KA, Gao Z, Fontaine N, Shu M, Fuller G, Smith DC, Nitschke DA, and Fagan RF. Epi Info™, a database and statistics program for public health professionals. CDC, Atlanta, GA, USA, 2011.) open-source calculator diagnostic test.

RESULTS

Total 145 cervical lymph node samples comprising 55 lymph node tissues and 90 aspirated fluids were received for the cervical TB diagnosis. GeneXpert CBNAAT was positive for MTB in 89 (61.37%) samples, whereas negative in 56 (38.62%) samples [Figure 1]. Depending on the cyclic threshold values, 31.46% (28) of samples were detected as very low, 49.43% (44) low, and 19.10% (17) medium. We did not find a high score in any sample. Thus, more than 80% of CBNAAT-positive samples were scored as "low" and "very low," suggesting a limited number of bacilli in the FNA sample [Figure 2]. In the study, 54 samples were of male patients, whereas 91 samples were from female patients. Higher positivity was noted in female patients. AFB was seen

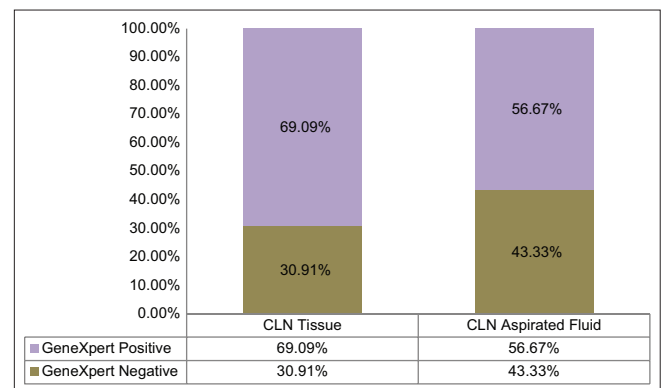


Figure 1: GeneXpert result for MTB detection (Sample wise). CLN: Cervical lymph node, MTB: *Mycobacterium tuberculosis*

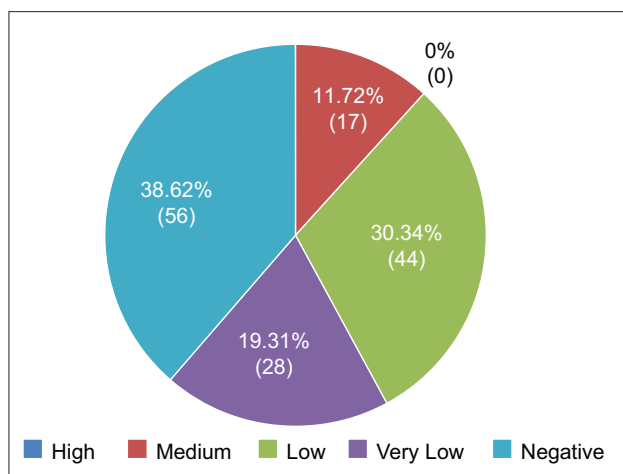


Figure 2: GeneXpert MTB detection result: Category-wise distribution based on CT value. CT: Cyclic threshold. High (<16 cycles), medium (16–22 cycles), low (22–28 cycles), and very low (>28 cycles)

in sputum smear of 6 (4.13%) patients, whereas chest X-Ray findings were suggestive of TB in 11 (7.5%) patients [Table 1].

On gross examination of lymph node aspirate, we observed purulent samples in 60% (54/90), caseous in 32.5% (29/90), and blood stained in 7.7% (7/90). CBNAAT GeneXpert rifampicin susceptibility report found that 78 (87.64%) samples were sensitive, whereas 7 (7.86%) were resistant and 4 (4.49%) were indeterminate.

Smear microscopy detected AFB in 31 out of 42 culture-positive and 3 out of 103 culture-negative cases, with AFB positivity of 23.4%. MTB were detected in 89 samples by using GeneXpert, whereas MTB was grown on culture from only 42 samples. When GeneXpert compared with culture, 36 samples were positive by both GeneXpert and culture, whereas 50 samples were negative by both the methods. However, 53 CBNAAT positive samples were negative on culture and 6 culture-positive samples found MTB not detected by CBNAAT [Table 2]. Hence, in the present study, for GeneXpert, we found sensitivity of 85.71%, specificity of 48.54%, PPV of 40.45%, and NPV of 89.28%. The percentage of agreement was 59% [Table 3].

DISCUSSION

TBL is the most common form of extrapulmonary TB, accounting for 30%–40% of TB cases [10]. Studies from the different parts of the country found variations. Chandigarh reported a high incidence of TBL (63.8%) in 2001. On the other hand, a study conducted in 2013 by Roy *et al.* in a tertiary care hospital of South India reported the incidence of TBL to be 18% [11]. Most of the time, diagnosis is confirmed by AFB using conventional microscopy, which is simple and rapid but lacks sensitivity or sensitivity varies depending on the source of the sample, whereas culture is more sensitive and specific but takes several weeks to get the results [12]. Moreover, AFB culture has an excellent detection rate in sputum samples, but the same cannot be said about lymph node samples [13]. Here, in this study, we observed only 28.93% culture-confirmed cases; this might be due to uneven distribution of bacilli and loss of viable bacilli

Table 1: Clinical characteristic of patient regarding sputum smear and chest X-ray findings in cervical lymph node *Mycobacterium tuberculosis* detected and *Mycobacterium tuberculosis* not detected patients

MTB results	MTB detected, n (%)	MTB not detected, n (%)	Total patients (145), n (%)
Conventional culture	42 (28.96)	103 (71.03)	145 (100)
GeneXpert	89 (61.37)	56 (38.62)	145 (100)
Sputum smear results			
Positive findings	5 (3.44)	1 (0.68)	6 (4.13)
Negative findings	84 (57.93)	55 (37.93)	139 (95.86)
Chest X-ray findings			
Positive findings	7 (4.82)	4 (2.75)	11 (7.58)
Negative findings	82 (56.55)	52 (35.86)	134 (92.41)

MTB: *Mycobacterium tuberculosis*

Table 2: Comparative analysis of GeneXpert and traditional culture method for cervical lymph node tuberculosis diagnosis

GeneXpert	Conventional culture test results		Total, n (%)
	MTB positive, n (%)	MTB negative, n (%)	
MTB positive	36 (85.71)	53 (51.45)	89 (61.37)
MTB negative	6 (14.28)	50 (48.54)	56 (38.62)
Total	42 (28.96)	103 (71.03)	145 (100.0)

MTB: *Mycobacterium tuberculosis*

Table 3: GeneXpert screening test evaluation for cervical lymph node tuberculosis diagnosis with compare to traditional culture method

Characteristics	Value (%)	Lower-upper (95% CI)
Sensitivity	85.71	72.16-93.28
Specificity	48.54	39.12-58.07
PPV	40.45	30.85-50.84
NPV	89.28	78.53-95
Agreement (kappa test)	59	
Diagnostic accuracy (CLN aspiration)	56.60	41.05-62.49
Diagnostic accuracy (CLN tissue)	63.63	43.90-71.16
Diagnostic accuracy (overall)	59.31	51.17-66.97

Method: Wilson score. Results from OpenEpi, Version 3, Open source calculator-diagnostic test. PPV: Positive predictive value, NPV: Negative predictive value, CI: Confidence interval, CLN: Cervical lymph node

during NALC-NAOH processing [14]. Recently, based on very low-quality evidence, the WHO also conditionally recommends Xpert to be used rather than conventional methods as the initial diagnostic test in patients suspected of having EPTB [6].

In the present study, sensitivity, specificity, PPV, and NPV of the GeneXpert test were 85.71%, 48.54%, 40.45%, and 89.28%, respectively. The percentage of agreement of the GeneXpert test result with culture was 59%.

The sensitivity of GeneXpert is 85.71%, which is well correlated with Tadesse *et al.* (sensitivity 87.8%) study at Jimma University specialized Hospital, in Southwest Ethiopia [15]. Penz *et al.*'s meta-analyses including 36 studies observed Xpert pooled sensitivity of 87% [16]. However, our finding is lower than what was found in a similar study by Ligthelm *et al.* (96.7%) [17] and Nur *et al.* (95%) [18].

According to a systemic review and meta-analyses conducted by Denkinger *et al.*, Xpert has a sensitivity ranging from 50% to 100% with a pooled sensitivity of 83% [19].

The specificity of GeneXpert in the current study is 48.54%; which is slightly similar to what was found by Nur *et al.* [18], but still lower in comparison to other studies mentioned above [15-17,19,20]. The presence of unrepresentative FNA specimens, scanty number of bacilli in the lymph node lesions, nonviable organisms due to the decontamination process, and the presence of amplified false-positive signals might all account for the reduced specificity. Again, the caseous lesion in the lymph node tissue may have contained dead tubercle bacilli. This can also be attributed to patients who were under antitubercular treatment when enrolled in the study.

Commonly used ZN microscopy has variable sensitivity depending on the source of the sample. In India, sensitivity ranges from 46% to 78% [21]. In the present study, we noted 23.4% AFB positivity. However, scanty AFB was observed in 80% of the smear-positive samples. This weak sensitivity can be explained by the paucibacillary nature of the specimens. Various other studies conducted over the years noted that AFB positivity ranges between 10% and 70% [11].

A female preponderance of 91 (62.75%) observed in the present study is correlated well with many other Indian studies [11]. Similarly, the maximum incidence of cervical lymphadenopathy was found in the second and third decade of the age, while rarely affecting patients in their extremes of age, this is found comparable with Mohapatra and Janmeja's study [22].

GeneXpert provides a semiquantitative grade (very low, low, medium, and high) on the basis of Ct value to each test positive for *M. tuberculosis* and these categories seem to be indicative of the bacterial load [23]. In the current study, we did not find a high score in any sample.

GeneXpert offers rapid detection of rifampicin-resistant MTB strains directly from the clinical samples; this is an added advantage over the smear microscopy and culture. In the present study, rifampicin resistance was identified in 7.9% (7/89) of the Xpert-positive cases. This rate was 4.7% in Ethiopian study [20]. Many previous studies reported 98%–100% agreement in the detection of rifampicin resistance strains using the Xpert test and phenotypic drug susceptibility test [24-27].

Considering the findings of agreement between GeneXpert and culture in the present study patients showing positive MTB result on GeneXpert, negative culture should be managed in conjunction with clinical presentation and radiological investigation. There were, however, few limitations of the present study. The incidence of pulmonary TB in patients with tuberculous lymphadenitis was not studied. It was not recorded whether the patients were suffering from any systemic illness at the time.

Our study and various other studies are suggested that in any region which is endemic or high prevalence, one can treat TB on the basis of strong clinical suspicion supported by laboratory, radiology, and histopathological examination [28].

CONCLUSION

Findings from the present study indicated the high sensitivity and low specificity; hence, we are trying to suggest that GeneXpert should be used with caution in nonrespiratory specimens. Clinical correlation is always required to establish the diagnosis. Further study will be needed to establish reliability of GeneXpert in lymphadenitis patients.

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Conflicts of interest

There are no conflicts of interest.

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