



Mediscope



The Journal of GMC

ORIGINAL ARTICLEdoi: <http://dx.doi.org/10.3329/mediscope.v6i1.38939>**Diagnosis of lymph node tuberculosis using the GeneXpert MTB/RIF in Bangladesh**TE Nur¹✉, AU Hosna², N Rayhan³, N Nazneen⁴**Abstract**

The purpose of this study was to evaluate the accuracy of the GeneXpert *M. tuberculosis* (MTB)/rifampicin (RIF) test for the detection of MTB in lymph node aspirated samples. This study was conducted in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Bangladesh. This study was done during the period from July 2013 to May 2015. A total of 317 clinically suspected tuberculous lymphadenitis patients without malignancy were included in the study. The culture test and GeneXpert test were used for detection of MTB in lymph node aspirated material. Among the 317 samples tested, the GeneXpert detected the DNA of MTB in 167 samples (52.7%), whereas culture test was positive in 74 (23.3%) specimens. GeneXpert also detected 8 RIF resistance cases. GeneXpert sensitivity and specificity results were assessed according to culture results. The sensitivity and specificity of the GeneXpert assay was 95.9% and 60.5%, respectively. The implementation of the GeneXpert MTB/RIF assay may dramatically improve the rapid diagnosis of lymph node TB. The GeneXpert MTB/RIF may replace usual conventional method like culture test for detection of MTB. **Key words:** *M. tuberculosis*, lymph node TB, rifampicin resistance, culture test, GeneXpert test.

Received: 20 July 2018

Accepted: 28 September 2018

Introduction

Tuberculosis (TB) remains a major public health problem in Bangladesh. Bangladesh ranks seventh in the list of the 30 high TB burden countries.¹ Accurate diagnosis and early treatment of TB has the potential to reduce morbidity and mortality associated with TB lymphadenitis (TBL). However, the differential diagnosis of TBL is broad and laboratory confirmation is the most important to guide appropriate therapy.^{2,3}

Cytology and conventional smear microscopy have been used as the initial diagnostic tools for TBL in resource poor settings.^{3,4} Fine needle aspiration cytology

(FNAC) is a simple and rapid diagnostic technique, but with low specificity because of the presence of similar cytological features in lesions other than those associated with TB.^{5,6} Conventional Ziehl-Neelsen (ZN) stained smear microscopy lacks sensitivity due to the paucibacillary nature of fine needle aspirates (FNA).⁷ Mycobacteriological culture and drug susceptibility testing are not always available in resource poor settings like Bangladesh.^{1,8} For these limitations more rapid and reliable methods are needed. In December 2010, World Health Organization (WHO) endorsed GeneXpert MTB/RIF (Cepheid, USA) for use in TB laboratories.⁹ The GeneXpert assay consists

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of a closed system that is based on real-time polymerase chain reaction (PCR). It can be used by operators with minimal technical expertise, enabling the diagnosis of TB and simultaneous detection of rifampicin resistance within 2 hours.⁹

The GeneXpert assay has been validated and optimized for sputum samples to diagnose HIV-associated TB and multidrug-resistant TB. WHO strongly recommends widespread use of GeneXpert for these groups of patients.^{10,11} More recently a number of studies were conducted to evaluate this assay using non-respiratory clinical samples from patients suspected of having extra-pulmonary TB (EPTB).^{8,12,13} In 2014, WHO has recommended GeneXpert over the conventional tests (including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having EPTB.¹⁴ However, this was a conditional recommendation due to very low-quality evidence available. More studies are therefore needed particularly in settings with high EPTB prevalence. Thus, we evaluated the performance of GeneXpert for the diagnosis of TBL using routinely collected FNA material.

The purpose of this study was to test the efficiency and reliability of the GeneXpert test for the detection of MTB bacteria in suspected lymph node TB aspirated material and to compare it to conventional culture method.

Materials and Method

Ethical clearance was first obtained from the Ethical Review Board, Bangabandhu Sheikh Mujib Medical University (BSMMU). The cross sectional study was conducted in collaboration with International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b).

All patients or guardians in case of children were requested for written consent prior to enrolment in the study. Any information concerning the patients was kept confidential. Laboratory results were reported back to

the physicians for treatment initiation or decision as early as available.

This study was conducted at a public tertiary care hospital in Dhaka, Bangladesh, where FNAC of lymph node lesions was done in the study subjects from July 2013 to May 2015. During this period, FNAC was performed on a total of 317 clinically suspected tuberculous lymphadenitis patients without having anti-TB drugs within 60 days of sample collection, malignancies, and inadequate sample (aspirated material) drawn.

Demographic and clinical information from the subjects were collected using a pre-tested questionnaire. The FNA sample was collected by a resident pathologist of the Department of Pathology, BSMMU.

Gross specimen appearance (caseous, purulent, and/or blood stained) was recorded at the time of specimen collection. The first one drop of the aspirates was used for cytomorphological diagnosis. Smears were fixed in 95% ethyl alcohol and stained with Papanicolaou staining. Another drop of specimen was used to make a smear for standard ZN staining. Both smears are examined by the same pathologist.

The cytological criteria for the diagnosis of TBL are based on the presence of the following cytomorphological appearances: epithelioid cell aggregate with or without Langhans giant cells and necrosis, epithelioid cell aggregate without necrosis, necrosis without epithelioid cell aggregate or polymorphonucleocytes with necrosis.¹⁵

The ZN stained smears were examined for the presence of AFB under oil-immersion (100x) using a light microscope. The remaining of the sample was processed for GeneXpert test conducted in the Department of Microbiology, BSMMU under the supervision of icddr,b, and for culture test done at the Tuberculosis Laboratory, icddr,b. Mycobacterial culture was done on Löwenstein-Jensen medium within 2 days of specimen collection.

Data were analyzed using the SPSS

software package (version 17). Sensitivity, specificity, positive and negative predictive values were calculated using culture for *M. tuberculosis* as a reference standard. Statistical analysis was done by Pearson *Chi*-square test and a *p* value <0.05 was taken as significant.

Results

The culture test revealed positive in 23.3% (Table 1). GeneXpert showed positive result in 167 patients (52.7%). Out of these, 71 cases were culture positive while 96 were culture negative (Table 1). Sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert were 95.9%, 60.5%, 42.6% and 98.0%, respectively; the percentage of agreement of GeneXpert with culture was 0.39 (Kappa test) (Table 2). GeneXpert also detected 8 RIF resistance cases.

Discussion

Based on very low quality evidence, WHO also conditionally recommends GeneXpert to be used rather than conventional methods as the initial diagnostic test in patients

suspected of having EPTB.^{11,14} In this study, the sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert test were 95.9%, 60.5%, 42.6% and 98.0%, respectively. The percentage of agreement of GeneXpert test results with culture test results was 0.39 (*Kappa test*) implying a slight agreement. The sensitivity of GeneXpert in the current study is similar what was found in a similar study by Ligthelm et al (sensitivity 96.7%).¹⁵ The specificity (60.5%) of the GeneXpert in the current study was found to be consistent with a previous study reported by Biadigilegn et al (specificity 69.2%).¹³ The specificity of GeneXpert test in this study was slightly lower in comparison to studies mentioned above. The presence of an unrepresentative FNA specimen, the scanty number of organisms in the lymph node lesions, nonviable organisms due to a decontamination process, and the presence of amplified false positive signals might all account for the reduced specificity.^{12,16}

In developing countries like Bangladesh, ZN stained smear microscopy is the only widely

Table 1. Association between the results of culture test and GeneXpert test

		Culture test results				Total	<i>p</i> value
		Positive		Negative			
		n	%	n	%	n	%
GeneXpert test results	Positive	71	95.9	96	39.5	167	52.7
	Negative	4	4.1	147	60.5	150	47.3
		74	23.3	243	67.7	317	100.0

n, number. *: Pearson *Chi*-square test.

Table 2. Some characteristics of GeneXpert test results

Characteristics	Value
Sensitivity	95.9%
Specificity	60.5%
Positive predictive value	42.6%
Negative predictive value	98.0%
Agreement (<i>Kappa test</i>)	0.39%

implemented method for quantifying the bacterial burden at the time of the initial diagnosis in FNA aspirated material. GeneXpert provides a semi-quantitative measurement of the number of MTB present in a sample. In this study, more than 90% of GeneXpert-positive samples were scored as 'low' and 'very low' suggesting a limited number of bacilli in FNA sample.¹⁷

FNA cytology as an inexpensive and reliable tool for TBL has been studied by a number of investigators.^{3,18,19} It is one of the most commonly used methods in resource poor settings. In the current study, the sensitivity and specificity of culture test compared to that of GeneXpert, was lower. To the best of our knowledge, information regarding the drug resistance pattern of mycobacterial strains isolated from TBL patients in Bangladesh is unavailable. GeneXpert test offers rapid detection of RIF resistant MTB strains directly from the clinical sample, an important advantage over culture test. Previous studies reported 98-100% agreement in detection of RIF resistance strains using the GeneXpert test and phenotypic drug susceptibility test.^{13,14,20,21} In this study, GeneXpert also detected 8 RIF resistance cases.

In conclusion, our findings indicated that GeneXpert MTB/RIF test is a useful tool for the detection of MTB with high sensitivity and specificity on FNA material with superior performance as compared to culture test. Besides improved sensitivity, the GeneXpert was able to identify patients with TBL due to RIF resistant TB. The GeneXpert test is an easy and suitable method to be used in TB endemic settings and its implementation could significantly improve the rapid diagnosis of TBL.

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Suggestion for citation of the above:

Nur TE, Hosna AU, Rayhan N, Nazneen N. *Diagnosis of lymph node tuberculosis using the GeneXpert MTB/RIF in Bangladesh. Mediscope 2019;6(1):19-23.*