Original Article

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Comparative evaluation of geneXpert MTB/RIF assay and Ziehl-Neelsen staining for the diagnosis of tuberculosis

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Abstract

Background: Ziehl-Neelsen (ZN) staining for the diagnosis of tuberculosis is frequently used. Since ZN smear technique has poor sensitivity, molecular tool like GeneXpert assay have recently been introduced in Nepal. This study compared GeneXpert MTB/RIF assay with ZN stain microscopy for the diagnosis of tuberculosis.

Objectives: To compare and evaluate GeneXpert MTB/RIF assay and Ziehl-Neelsen staining for the diagnosis of tuberculosis.

Methods: An analytical observational study was conducted in Manipal Teaching Hospital, Pokhara from February 2020 to August 2021 after ethical clearance. A total of 355 clinical specimens (Sputum, Broncho-alveolar lavage, pus, lymph node aspirate etc) were collected and processed for Zeihl-Neelson staining and GeneXpert assay as per the recommended guidelines.

Results: Zeihl-Neelson staining detected acid-fast bacilli (AFB) in 17 (4.78%) specimens, while GeneXpert assay showed positivity in 37 (10.42%). All the 17 smear positive sputum samples yielded positive result by GeneXpert also. AFB were not detected in any of the 318 samples negative by GeneXpert. Fifteen (83.3%) of the 18 specimens that showed high to medium density of bacilli by GeneXpert were positive by Ziehl-Neelsen staining. Only two (10.5%) of the 19 specimens with low bacillary density by GeneXpert were positive by Ziehl-Neelsen Staining. These differences were statistically significant ($p < 0.001$). Out of 37 positive specimens, one yielded rifampicin resistant *Mycobacterium tuberculosis*.

Conclusion: ZN smear, though, rapid, lacks sensitivity. GeneXpert, on the other hand, can be relied upon, as it detected significantly higher number of cases, demonstrated bacillary density and drug resistance.

Key words: Microscopy; *Mycobacterium tuberculosis*; Polymerase chain reaction; Sputum.

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INTRODUCTION

Tuberculosis (TB) is a treatable condition, still it remains one of the biggest challenges for developing countries.[1] Over and above, recent upsurge of drug resistant TB cases globally, including multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) raised concern over early detection of case and the appropriate management.[2]

Diagnosis of TB, is largely dependent on Ziehl-Neelsen (ZN) staining of clinical samples in developing and underdeveloped countries because of its low cost. Culture for the detection *Mycobacterium tuberculosis* (*M. tuberculosis*) remains the gold standard for TB diagnosis, but it is time consuming and requires the setup of high-quality laboratory. On the contrary, microscopy, though simple, rapid, and cost effective, has limited sensitivity and this, alone may not accurately diagnose TB. Newer tools like geneXpert MTB/RIF assay, a polymerase chain reaction (PCR) based molecular assay, is now

widely available and used in Nepal. It can detect both *M. tuberculosis* complex deoxyribonucleic acid, and genetic mutation associated with rifampicin resistance simultaneously in less than two hours' time.

The present study was, therefore, conducted to compare the efficacy of GeneXpert assay with ZN staining for the diagnosis of TB in a tertiary care hospital in Nepal.

METHODOLOGY

This was an analytical observational study conducted between February 2020 to August 2021 in the department of Microbiology, Manipal Teaching Hospital in Western Nepal, after obtaining approval from the institutional research committee (IRC) of Manipal (Ref. MEMG/470/IRC).

Patients clinically suspected to have Pulmonary TB with symptoms such as cough with or without expectoration for more than two weeks, weight loss, fatigue, haemoptysis, loss of appetite, and those with extrapulmonary manifestations in the form of chronic meningitis, peritonitis or lymphadenitis, either admitted to the hospital, attending the directly observed therapy short course (DOTS) centre or peripheral health centre (PHC) were included in the study.

A total of 355 samples were collected by consecutive sampling technique. The sample size was derived using formula sample size N=z^2 P(1-P)\d^2, where N= sample size, Z= level of confidence (1.96), P= expected prevalence, here in this study P = 0.2059 (20.59%)[5], d= precision (0.05) and minimum sample size obtained was 251. Duplicate specimens from single patient were considered as a single specimen. Sputum samples mixed with blood, food particles, not enough volume were not processed. The samples comprised of 335 sputum samples; 12 bronchoalveolar lavage (BAL) fluid; five CSF and one each of peritoneal fluid, pus, and lymph node aspirate.

Morning sputum specimens, were collected from the patients after proper instructions so as to get ideal sample in a falcon tube of 50 ml capacity. At least 5 ml of sputum samples were collected from each patient. In case of in-patients who were unable to provide samples, sputum production was induced by nebulisation with hypertonic saline or BAL fluid was collected according to discretion of the clinician. CSF and other body fluids were collected by adopting standard recommended procedures.[6] All samples were sent to the department of Microbiology, Manipal Teaching Hospital (MTH) without delay.

A direct smear was prepared on a clean grease free glass slide using a clean disposable wooden applicator stick. The smear was air dried, heat fixed and stained with ZN staining method as per the revised national tuberculosis control program (RNTCP) guidelines.[7] Acid fast bacilli were seen as bright red/pink rods against blue background.

GeneXpert assay procedure adopted, was in accordance with the WHO recommended guidelines.[8] About three ml of the specimen was mixed with twice its volume of sample reagent. The mixture was then vortexed and incubated at room temperature for 10 minutes. Thereafter it was again vortexed and incubated for another five minutes. About two ml of this processed sample was then added to GeneXpert cartridge which was then loaded in the machine. The results were finally interpreted by the GeneXpert system based on fluorescent symbols which was displayed on the system monitor after about two hours.

The data were collected, entered and analysed using SPSS Statistics for Windows, version 21.0 (SPSS Inc., Chicago, Ill., USA). Categorical variables were calculated as percentages. Chi-square test was used to compare two groups. All p-values <0.05 were considered as statistically significant.

RESULTS

In total, 355 clinical samples were processed for GeneXpert assay and ZN staining. These included 335 sputum samples, 12 BAL fluids, five CSF samples and one each of pus, peritoneal fluid and lymph node aspirate.

GeneXpert method yielded positive results in 10.42% (37/355) of the samples (Table 1). It was noteworthy that high prevalence rates (ranging between 11.36-18.6%) were observed among adolescents and adults in the age group of 10-40 years and also among elderly people who were above 60 years of age (Table 2). The ZN staining showed acid fast bacillus only in 4.78% (17/355) samples (Table 3).

Out of the 37 GeneXpert positive yields, 35 were from sputum samples and the remaining two were from BAL fluids. *Mycobacterium tuberculosis* (MTB) was not detected in any of the other specimens like CSF, pus, peritoneal fluid or lymph node aspirate (Table 1).

There was a good concordance between the mycobacterial detection in GeneXpert assay and the ZN staining findings (Table 3). Fifteen (83.3%) of the 18

specimens that showed high to medium density of bacilli by GeneXpert were positive by ZN staining. Contrary to this, only 2 (10.5%) of the 19 specimens with low and very low bacillary density by GeneXpert were positive by ZN smear examination. In remaining 17 specimens, AFB were missed by ZN staining but detected by GeneXpert Assay. These differences were found to be statistically significant (p<0.001). Acid fast bacilli were detected from 17 sputum specimens all of which were detected by GeneXpert also. No AFB were detected in non-sputum specimens. AFB were not detected in any of the 318 samples negative by GeneXpert. This difference, too, was found to be statistically significant (Table 4, p-value <0.001).

Table 1: GeneXpert assay results from various samples received during the study period

	GeneXpert positive (%)	GeneXpert Negative (%)	Total
Sputum	35 (10.44)	300 (89.55)	335
BAL	2 (16.66)	10 (83.33)	12
CSF	-	5 (100)	5
Peritoneal fluid	-	1 (100)	1
Pus	-	1 (100)	1
Lymph-node aspirate	-	1 (100)	1
Total	**37 (10.42)**	**318 (89.57)**	**355**

Table 2: Prevalence of TB using GeneXpert in relation to age group of patients

Age	GeneXpert +ve (%)	GeneXpert –ve (%)	Total
0-10	-	12 (100)	12
11-20	8 (18.60)	35 (81.39)	43
21-30	7 (13.20)	46 (86.79)	53
31-40	7 (13.46)	45 (86.53)	52
41-50	5 (11.36)	39 (88.63)	44
51-60	2 (4)	48 (96)	50
61-70	5 (10)	45 (90)	50
>71	3 (5.88)	48 (94.11)	51
Total	**37 (10.42)**	**318 (89.57)**	**355**

Table 3: Density of *M tuberculosis* (MTB) detected by GeneXpert as compared to ZN smear positivity

Density of MTB in eneXpert	ZN staining		
	AFB seen	AFB not seen	Total
High	4 (80)	1(20)	5
Medium	11(84.61)	2(15.38)	13
Low	2(13.33)	13(86.66)	15
Very low	-	4 (100)	4
Total	**17 (45.94)**	**20 (54.05)**	**37**

Table 4: Correlation of GeneXpert and ZN staining positivity

GeneXpert	ZN positive	ZN negative	Total	
Positive (37)	17*	20	37	*p <0.001
Negative (318)	0*	318	318	
Total (355)	**17**	**338**	**355**	

DISCUSSION

Early diagnosis of TB is necessary in order to break the disease transmission events by advocating appropriate and timely anti-tubercular treatment. Although ZN smear positive patients in general are considered to be infectious, yet smear negative symptomatic cases also do have the potential to transmit the disease.[9] However, smear microscopy has low sensitivity.[10] Thus molecular techniques like GeneXpert system have been introduced into practice recently with better sensitivity and specificity.[11]

In the present study, GeneXpert positivity for the MTB remained to be 10.42% (37/355) as against smear positivity in only 4.78% (17/355) of the samples. Munir et al. reported smear positivity in 67.5% of the samples and GeneXpert positivity among 77.4% samples tested.[12] Others however, found that 23.73% of the specimens were positive by ZN smear and 34.24% by GeneXpert assay.[13] The reason for low rate of positivity both by ZN smear examination and by GeneXpert in the present study as compared to others, could be because of the fact that the samples we processed, were inclusive of those obtained from community screening, contact tracing along with clinical samples from patients reported to hospital.[12-14] However, the findings from this study were comparable to the findings of Mechal in the context of high yield of MTB by GeneXpert assay as compared to the smear test.[3] Hence, it is needless to emphasise that GeneXpert being a molecular tool overcomes the limitations of smear microscopy which mainly depends upon factors like technical expertise in microscopy, and bacterial load in a particular sample.

In addition to the superiority of GeneXpert assay over conventional microscopy in detecting MTB (10.42% vs 4.78%), from both pulmonary and extrapulmonary TB cases, this study highlighted that samples reported negative in smear examination could be picked up by GeneXpert method. Similar observation was done by Umair et al., where out of total 50 GeneXpert positive samples, ZN staining was positive only in 30 samples.[15]

As shown in Table 4, a total of 20 (54.05%) of 37 samples which were negative by ZN smear were found to be positive for MTB by the GeneXpert assay. This observation of current study is in agreement with the findings of other workers.[10] Over and above, we also observed a statistically significant correlation between GeneXpert positivity and ZN smear positivity. Notwithstanding the low positivity of ZN smear, this technique cannot be totally ignored. Besides being rapid and user-friendly tool, its results were found to be in good agreement with the density of mycobacterial yield detected by GeneXpert. It was noteworthy that majority (15, 83.33%) out of 18 samples showing high yield of bacilli in the GeneXpert, were positive by the ZN smear too.

Overall, GeneXpert, in addition to being superior over smear examination, has therapeutic implications as well. Choice of treatment regimen of tuberculosis depends on the identification of drug resistance pattern of the organism. Presently, the global rate of MDR tuberculosis accounts for 3.3% of total new cases. Treatment success rate is also less which is 57% in MDR tuberculosis.[3] In these perspectives, GeneXpert test can differentiate MDR/Rifampicin resistant tuberculosis on the same day and guide us to initiate proper therapeutic regime.[16] In the present study, a single specimen (2.7%) out of the 37 positive samples had yielded rifampicin resistant/ MDR isolate. The patient was a 69-year-old diabetic male having no history of past tuberculosis infection. The patient had marked clinical improvement with the second line of anti-tubercular treatment (ATT).

CONCLUSION

GeneXpert depicted significantly higher level of detection of MTB as compared to ZN smear examination, even in cases with low to very low bacillary load in the clinical samples. It also differentiated MDR/rifampicin resistance on same day, which is very important to initiate the treatment. Thus, GeneXpert assay should be strongly advocated in smear negative, but clinically suspected cases of tuberculosis.

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