ORIGINAL ARTICLE

GeneXpert and Acid-Fast Bacilli Smear for Diagnosis of Pulmonary Tuberculosis in a Tertiary Care Center of Rural Nepal.

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ABSTRACT

Introduction: Mycobacterium tuberculosis is the second most infectious cause of death in adults globally and is major health trouble in Nepal. The diagnosis of pulmonary tuberculosis is mainly carried by acid-fast bacilli smear microscopy, culture on Lowenstein–Jensen media, and GeneXpert MTB/RIF assay. This study aimed to evaluate the GeneXpert MTB/RIF and Acid-Fast Bacilli stain smear used for the diagnosis of pulmonary tuberculosis.

Methods: A cross-sectional study was performed at Karnali Academy of Health Sciences, Jumla, from July 2021 to June 2022. Our study population comprised 390 probable cases of pulmonary tuberculosis from the ages of 2 to 92 years. A total of 390 sputum specimens from suspected presumptive tuberculosis sufferers were tested using the direct smear staining technique of Acid-Fast Bacilli stain smear and GeneXpert for the detection of MTB/RIF. The GeneXpert MTB/RIF technique was compared with the Acid-Fast Bacilli stain smear. Patient's demographic details, sputum AFB smear, and GeneXpert test results were collected and entered in working Performa. Data were analyzed using SPSS 16.0.

Results: Out of 390 samples 13.84% were positive for Mycobacterium tuberculosis via GeneXpert MTB/RIF assay while 5.12% were positive through AFB microscopy which was statistically significant ($p<.0001$). When compared with GeneXpert, taking GeneXpert as the gold standard in the rural setup of Nepal the sensitivity of AFB staining was 37.03%, while its specificity was 90.81%.

Conclusions: GeneXpert MTB/RIF is a rapid test that can aid in the timely diagnosis of Mycobacterium tuberculosis, facilitating the timely treatment in the rural area.

INTRODUCTION

Tuberculosis (TB) remains an international health burden, especially in developing countries. In 2019, an estimated 10 million people fell ill with TB worldwide, and a total of 1.4 million people died from TB (including 208 000 people with Human Immunodeficiency Virus). Global, TB is one of the pinnacle 10 causes of death and the leading cause of a single infectious agent (above HIV/AIDS).[1] Primarily on a recent National TB Prevalence Survey in 2018, there are about 117,000

humans residing with TB in Nepal resulting in a reported prevalence rate of 416 per 100,000 population, 1.8 times higher than previously envisioned.[2] In 2018, there were 69,000 people who fell sick with TB in Nepal, however, only 32,474 (47%) were reported to the National TB Program.[3]

It is a chronic, granulomatous, air-born bacterial infectious disease that can involve the lung and other organ systems. Poverty and malnutrition aggravate the weight of TB. The primary microbiological agent is Mycobacterium tuberculosis in the vast majority of instances. The disease may transform into the active phase in 10% of cases and the lungs are the most common place of involvement.[4] Pulmonary TB is the most common presenting form and the form with the highest transmission rates.[5]

Mycobacterial culture is the well-known standard test for the diagnosis of TB, and it can detect as low as 10 bacilli/ml of the specimen, but it is time-consuming and requires specialized laboratory infrastructure. A GeneXpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) assay (a nucleic acid amplification test) is a sensitive but expensive test for the diagnosis of TB with a lower detection threshold of 136 bacilli/ml of the specimen. Additionally, it also detects the presence of rifampicin resistance, which is a surrogate marker of multidrug-resistant (MDR) TB. However, the GeneXpert MTB/RIF assay costs 10 times more than the Lowenstein-Jensen (LJ) culture and 20 times as compared to Acid-Fast Bacilli stain smear microscopy. AFB smear microscopy is an inexpensive but less sensitive test for the diagnosis of TB with a lower detection threshold of 5,000 bacilli/ml of the specimen.[6]

The main objective of the current study is to evaluate the GeneXpert MTB/RIF and AFB stain smear used for the diagnosis of pulmonary tuberculosis in a tertiary hospital in the rural part of Nepal. Karnali Academy of Health Sciences is located in the rural part of Nepal, because of the lack of specialized laboratory infrastructure mycobacterial culture is not started yet, however, it will be stared soon as we are developing infrastructure.

METHODS

Study design: This is a cross-sectional study conducted in the laboratory department of Karnali Academy of Health Sciences Teaching Hospital, Jumla. This study included one-year data of sputum samples collected from 390 notified/suspected TB patients tested for AFB smear microscopy and GeneXpert during the period July 2021 to June 2022.

Inclusion criteria: Suspected TB patients from all age groups who had sputum samples and had available recorded test results for AFB test and GeneXpert were included in the study.

Exclusion Criteria: Patients with extrapulmonary TB, sputum samples less than 3 ml, and invalid and error results were excluded from the study.

A total of 390 individuals of all ages who were clinically diagnosed as suspected TB patients were included in the study. The portion of sputum samples from routine AFB tests and GeneXpert were collected. Acid-fast microscopy was done using a sterile application stick, a drop of raw sputum was placed on a grease-free glass slide and evenly spread, dried, and heat fixed. Thereafter, AFB staining was done according to previously described methods.[7]

GeneXpert test was performed according to the manufacturer's instructions. Briefly, with the aid of a sterile pipette, 2 ml of GeneXpert reagent was added to 1 ml of sputum sample and incubated at room temperature for 15 min. The mixture was agitated twice at 5 min intervals. The liquefied mixture was transferred into the GeneXpert cartridge using a sterile pipette and loaded into the GeneXpert instrument. Results were available within 2 hrs.

Taking GeneXpert as the Gold standard, the sensitivity, and specificity of AFB smear assay were calculated by the following formula.[8]

Sensitivity = True positive (TP)/True positive (TP) +False Negative (FN) X 100

Specificity= True Negative (TN)/True Negative+ False Positive (FP) X 100

Ethical Approval was taken from the Institutional Review Committee, Karnali Academy of Health Sciences "IRC-KAHS" (reference Number: 2078/2079/34). Valid and reliable data were collected at the laboratory of Karnali Academy of Health Sciences, Teaching Hospital Jumla. Confidentiality of the patient information was assured by maintaining privacy and not recording personal information from the record book.

Patient demographic details, sputum AFB smear microscopy, and GeneXpert test results were collected for data analysis. The findings were recorded in the proforma, then transferred to a spreadsheet program and data were analyzed in SPSS 16.00 using descriptive statistics, and

required useful tests were applied.

RESULTS

Out of 390 patients tested, the percentage of males tested was 55.90 and the prevalence rate among males is 5.05%. A maximum of up to 20% of the males in the age group between 20-29 were positive for Ziehl-Neelsen. On the other hand, the percentage of females tested was 44.10 and the prevalence rate among females is 5.23%. Among the females who were positive for AFB, 15% were in the age group 20-29 years.

Table 1. Prevalence of Tuberculosis using AFB in relation to age groups and gender

Age groups years	Male tested	Positive number (%)	Female tested	Positive number (%)
1-10	15	0	6	0
11-19	18	0	14	1(5)
20-29	48	4(20)	22	3(15)
30-39	18	2(10)	29	2(10)
40-49	15	0(0.0)	2	1(5)
50-59	30	2(10)	25	1(5)
60-69	39	2(10)	28	0
70-79	26	1(5)	26	1(5)
80-89	9	0	1	0
90-99	0	0	1	0
Total	218	11	172	9

Out of 390 patients tested, the percentage of males tested was 55.90 and the prevalence rate among males is 12.84. Of the maximum males, 22.22% were positive on GeneXpert within the age range of 20-29 years, on the other hand, the percentage of females tested was 44.10 and the prevalence rate is 16.86. Female patients positive with MTB/RIF GeneXpert were 2 (3.70%) for the ages of 11-19 years, 10 (18.51%) for the ages of 20-29 years, and 4 (7.40%) for age groups 40-49.

Table 2. Prevalence of tuberculosis using Gene expert MTB/RIF in relation to age groups and gender in positive cases

Age groups	Males tested	Number positive (%)	Female tested	Number positive (%)
1-10	15	0(0.0)	6	0(0.0)
11-19	18	3(5.55)	14	2(3.70)
20-29	48	12(22.22)	22	10(18.51)
30-39	18	2(3.70)	29	4(7.40)
40-49	15	3(5.55)	20	3(5.55)
50-59	30	4(7.40)	25	3(5.55)
60-69	39	2(3.70)	28	1(1.85)
70-79	26	2(3.70)	26	3(5.55)
80-89	9	0	1	0
90-100	0	0	1	0
Total	218	28	172	26

Table 3 shows patients who tested negative for tuberculosis using the AFB technique tested positive for MTB/RIF GeneXpert technique. The table also shows age distribution in relation to positive cases for MTB/RIF GeneXpert. 20 out of 20 AFB technique positives were GeneXpert technique positive, while 34 out of 370 ZN techniques negative were detected positive by GeneXpert technique.

Table 3. AFB negative and MTB/RIF GeneXpert positive cases

Age groups (years)	Number tested	AFB Negative	MTB/RIF GeneXpert Positive
1-10	21	0	0
11-19	32	0	4
20-29	70	0	15
30-39	47	0	2
40-49	35	0	5
50-59	55	0	4
60-69	67	0	1
70-79	52	0	4
80-89	10	0	0
90-100	1	0	0
Total	390	0	34

Among all the 390 patients, who were tested both for GeneXpert and AFB, their result was significantly different from each other (p<0.001). It also showed that taking GeneXpert as the gold standard, the sensitivity of AFB staining is only 20/(20+34) = 37.03%, while its specificity is 336/336+34 = 90.81%

Table 4. Comparative analysis of AFB stain and GeneXpert techniques for the diagnosis of tuberculosis

Test	Results		Chi Square	P value
	Positive	Negative	17.259	.000033
GeneXpert	54 (13.85%)	336 (86.15%)		
AFB Stain	20(5.12%)	370 (94.88%)		
Total	74	706		

DISCUSSION

Diagnosis of tuberculosis remains a challenge due to various factors like the paucibacillary nature of mycobacterium, inadequate specimens and resemblance of clinical symptoms of diseases with many other respiratory ailments. [9]

In our study, we tried to compare the ability of AFB smear (Ziehl-Neelsen) in the detection of Pulmonary TB as compared to GeneXpert at Karnali academy of health sciences.

From the study, the prevalence of M. tuberculosis with the Ziehl-Neelsen technique in selected patients was found to be 5.12%. The prevalence rate among males is 5.05%, while among females is 5.23%. There was a general increase in the prevalence of tuberculosis as the age progressed with the age group between 20 and 39 recording the highest proportional distribution (25 %). This is consistence with the existing knowledge about host factors associated with TB. In a previous study by Bagchi *et al in* Mumbai, similar results were found.[10] This age group is most likely to come in contact with other non-infected people, thereby augmenting the risk of the spread of infection.[11] This highlights the need for strict airborne infection control measures in healthcare settings and other public places.

In our study, the yield of GeneXpert in suspected (probable TB) cases was 13.84% while AFB smear yielded 5.12% which was statistically significant (p<.0001). The GeneXpert result shows a large number of patients diagnosed with tuberculosis. This might be because the AFB is not present in the initial few days because most of the patient has visited within less than 5 days of the appearance of the cough. A similar study carried out by Shagufta Iram et al[1] shows the GeneXpert MTB/RIF is a sensitive method for rapid diagnosis of tuberculosis, especially in smear-negative cases and in PTB as compared to the conventional ZN staining. For countries endemic for TB GeneXpert can serve as a sensitive and time-saving diagnostic modality for pulmonary TB. Our study is consistent with other studies which have suggested the benefit of GeneXpert in smear-negative patients in developing countries.[13,14] Its place in the

clinical diagnostic algorithm in national health programs needs exploration. GeneXpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing AFB smear microscopy and other molecular methodologies in the TB-endemic region. To the best of our knowledge, this is the first comparative study to report tuberculosis infection using the GeneXpert technique in the Karnali zone. Similarly, studies conducted in Pakistan by Ullah et al have shown a high yield of GeneXpert as compared to AFB smear.[15]

Sputum smear light microscopy, the patient's symptoms combined with CXR results is the preferred algorithm for TB diagnosis in Nepal. A smear test is neither relevant nor sensitive enough for the diagnosis of TB alone and needs additional diagnosis as well as clinical suspecting to decide either patients should be enrolled on the antibiotic or not.[16]

CONCLUSIONS

The microscopy (Ziehl-Neelsen) and GeneXpert in laboratory diagnosis of pulmonary tuberculosis were compared. MTB RIF positivity among sputum-negative cases is large in this study. The integration of molecular techniques such as GeneXpert in the final identification of M. tuberculosis complex/M. tuberculosis is recommended as this can provide timely intervention in the diagnosis of tuberculosis and treatment. GeneXpert MTB/RIF is a rapid test that can aid in the timely diagnosis of TB, facilitating the timely treatment The Government of Nepal Ministry of Health and population should implement GeneXpert in each DOTS Centre located in rural area of Nepal for better diagnosis.

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