ORIGINAL ARTICLE

GENEXPERT OF CERVICAL LYMPH NODE SPECIMENS IN THE DIAGNOSIS OF EXTRAPULMONARY TB AND RIFAMPICIN RESISTANCE

Niaz Hussain Soomro¹, Saifullah Baig², Nisar Rao ³, Aneeqa Ahsan Zafar¹, Farwa Hashmat⁴, Sattar Bakhsh Awan⁵, Hashmat Ullah⁶
¹Department of Thoracic Surgery, ²Department of Drug Resistant TB and ³Department of Chest Medicine, OJHA Institute of Chest Diseases, Dow University of Health Sciences Karachi, ⁴Department of Chemistry and ⁵Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan

ABSTRACT

Background: Tuberculosis is rapidly becoming a global disease. The objective of the study was to determine the yield of the GeneXpert in the detection of DNA of mycobacterium tuberculosis and rifampicin resistance in cervical lymph node tissue specimens.

Material & Methods: This cross-sectional study was conducted at the departments of Chest Medicine and Thoracic Surgery at OJHA Institute of Chest Diseases, Dow University of Health Sciences Karachi, Pakistan, from April 2014 to June 2015. The sample size was 54, selected through non-probability consecutive sampling technique. All patients who presented with cervical lymph adenopathy were included in the study. Demographic variables were; gender and age in years. Gender was nominal whereas age was numeric variable. Research variables were; DNA of mycobacterium TB, Rifampicin resistance. Both were nominal having attributes of positive and negative each. Data was collected and analyzed descriptively as percentages and mean along with range depending upon the data type being categorical or numeric. Descriptive statistic were calculated using SPSS Version 15.

Results: Out of 54 patients, 23(42.50%) were males and 29(53.70%) females. The mean age of the patients was 32.3 years with range of 75 years (8- 83 years). Among the 54 samples tested, the GeneXpert detected the DNA of mycobacterium tuberculosis in 40 samples (74%), whereas 14(26%) were found negative. Out of 40, GeneXpert positive patients, Rifampicin resistance was detected in 2 patients (5%). And 38(95%) were Rifampicin sensitive. One of those 2 patients did not have any prior history of anti-tuberculosis therapy and was diagnosed with primary multidrug resistant tuberculosis where as the other patient being diagnosed with tuberculosis previously and completing a course of anti-tuberculosis therapy.

Conclusion: GeneExpert MTB/RIF is an efficient method to detect mycobacterium TB along with Rifampicin sensitivity in cervical lymph node tissue specimens.

KEY WORDS: Tuberculosis; Rifampicin; Mycobacterium; Diagnosed; Tissue.


INTRODUCTION

According to statistics released by WHO, every year there are approximately nine million new cases of tuberculosis and annually 2 million people die because of tuberculosis.¹ Of the 22 countries in the world with a high prevalence of tuberculosis, Pakistan ranks 5th. The prevalence of all types of TB in Pakistan is 342 per 100,000 population and incidence of 275 new cases per 100,000 each year.² The spread of tuberculosis is largely dependent on the socioeconomic and hygienic conditions that is why this disease is more common in 3rd world countries like India, Bangladesh and Pakistan. According to a survey, M. tuberculosis infects 44% of the population.³ TB can affect virtually any organ of the body. Twenty percent of all TB cases are attributed to being
extra pulmonary with lymph nodes being the most common site of occurrence. Existing tests for the diagnosis of extra pulmonary tuberculosis are limited in accuracy and time of diagnosis, and often require invasive procedures and special expertise. WHO recommends that Gene Xpert/RIF assay should be used as the diagnostic test of choice for TB endemic areas. The Xpert MTB/RIF assay is a rapid PCR test for pulmonary Tuberculosis detection (sensitivity 89%, specificity 99%). Raj reported that this test should be amongst the major milestones for TB diagnosis and hence the management options. The Xpert MTB/RIF detects DNA sequences specific for mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction. It is based on the Cepheid GeneXpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). The Xpert MTB/RIF purifies and concentrates mycobacterium tuberculosis bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process identifies all the clinically relevant rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the mycobacterium tuberculosis genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required to operate. This test was developed as an on-demand near patient technology which could be performed even in a doctor’s office if necessary. The objective of the study was to determine the yield of the GeneXpert in the detection of DNA of mycobacterium tuberculosis and rifampicin resistance in cervical lymph node tissue specimens.

MATERIAL AND METHODS

This cross-sectional study was conducted at the departments of Chest Medicine and Thoracic Surgery at OJHA Institute of Chest Diseases, Dow University of Health Sciences Karachi, Pakistan, from April 2014 to June 2015. The sample size was 54, selected through non-probility consecutive sampling technique. All patients who presented with cervical lymph adenopathy were included in the study.

After well informed and written consent, lymph node specimens were collected from all patients and sent to the bacteriology laboratory where the biopsy samples were crushed in a sterile mortar and then used for the GeneXpert test. The test consists of two main parts. (i) The Xpert MTB/RIF cartridge which contains liquid sample-processing and PCR buffers and lyophilized real-time PCR reagents and (ii) the GeneXpert instrument, which controls intracartridge fluidics and performs real-time PCR analysis. The reagent was added in the ratio of 2:1 to the specimen. During a 15-min incubation period at room temperature, the container is shaken twice. 2ml of the inactivated mixture was transferred to the Xpert test cartridge. The loaded assay cartridge is placed into the GeneXpert instrument bay and the mycobacterium tuberculosis automated detection protocol method is selected from the included software. The Xpert MTB/RIF assay’s data analysis algorithm identifies a sample as mycobacterium tuberculosis positive if at least two of the five rpoB probes are positive within two cycles of each other. Samples that were negative for mycobacterium tuberculosis are reported as mycobacterium tuberculosis negative. Rifampin resistance was detected by the failure of one or more of the rpoB-specific molecular beacons to hybridize properly to the rpoB amplicon. This was defined by the presence of greater than a 3.5-cycle difference in the cycle threshold values between the earliest and latest rpoB probe signals or if one to three rpoB probes did not produce any measurable signal. The whole process takes about 90 minutes to complete. Demographic variables were; gender and age in years. Gender was nominal whereas age was numeric variable. Research variables were; DNA of mycobacterium TB, Rifampicin resistance. Both were nominal having attributes of positive and negative each. Data was collected and analyzed descriptively as percentages and mean along with range depending upon the data type being categorical or numeric. Descriptive statistics were calculated using SPSS Version 15.

RESULTS

Out of 54 patients, 23(42.50%) were males and 29 (53.70%) females (Fig-1).

The mean age of the patients was 32.3 years with range of 75 years (8- 83 years).

Among the 54 samples tested, the GeneXpert detected the DNA of mycobacterium tuberculosis in 40 samples (74%), whereas 14(26%) were found negative (Fig-2).

Out of 40, GeneXpert positive patients, Rifampicin resistance was detected in 2 patients (5%). And 38(95%) were Rifampicin sensitive (Table-1). One of those 2 patients did not have any prior history of anti tuberculosis therapy and was diagnosed with primary multidrug resistant tuberculosis whereas the other patient being diagnosed with tuberculosis previously and completing a course of anti tuberculosis therapy.
Fig 2: Distribution of DNA of mycobacterium TB detection in cervical lymph node study by GenXpert (n=54)

Table:1 distribution of rifampicin resistance in cervical lymph node tissue study by GeneXpert (n=40)

<table>
<thead>
<tr>
<th>Rifampicin resistance</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>2</td>
<td>(5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>38</td>
<td>(95%)</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>

DISCUSSION

Tuberculosis is a common disease in Pakistan which is considered to be a major cause of healthcare expenditure. On the basis of WHO 2014 statistics, the incidence of TB in Pakistan is approximately 275/100,000 people and continues to increase every year due to the lack of proper resources and knowledge amongst patients regarding its treatment. In the current situation, it is necessary to improve the diagnostic tools for TB. Diagnosis of lymph node tuberculosis is difficult despite of several different available investigations.

Microscopy is the easiest and cheapest way to diagnose tuberculosis. Its sensitivity depends on the prevalence of TB in the area. The sensitivity ranges from 46-78% in India. Culture for AFB is the gold standard method for confirmation of TB but it takes several weeks for the results to come in, making it a less ideal investigation in the diagnosis of TB. Moreover, AFB culture has an excellent detection rate in sputum samples but the same cannot be said about lymph node samples unfortunately. For extrapulmonary tuberculosis, the World Health Organization (WHO) suggests that smear microscopy be replaced with Xpert MTB/RIF assay. The Xpert MTB/RIF assay performs real-time PCR and requires minimal technical expertise, enabling the rapid diagnosis of TB as well as detection of rifampicin resistance. The results are generally available within 2 hrs. The sensitivity of Xpert MTB/RIF assay in sputum has been shown to be 98.2% in patients who are smear-positive, culture positive patients. In smear-negative, culture positive patients, the sensitivity is 72.5%. In a study conducted in Tunisia, GeneXpert test conducted on lymph node tissue samples was positive in 77% of the patients. In our case, GeneXpert was positive in 40/54, 74% of the lymph node tissue samples tested.

The rpoB gene mutation is present in 95%–99% of RIF-resistant isolates and Xpert/RIF assay is able to quickly detect it. In addition to rpoB gene mutations, this test is also able to detect mutations in katG and inhA which result in INH resistance. In our study, 2 of the 40 samples that test positive for MTB on GeneXpert, showed rifampicin resistance. One of those 2 patients did not have any prior history of tuberculosis or taking anti tuberculosis therapy and was diagnosed as a case of primary multi drug resistance tuberculosis whereas the other patient was diagnosed with tuberculosis previously and completing a course of anti-tuberculosis therapy.

CONCLUSION

GeneXpert MTB/RIF is an efficient method to detect mycobacterium TB along with Rifampicin sensitivity in cervical lymph node tissue specimens.

REFERENCES

8. Van Kampen SC, Anthony RM, Klater PR. The realistic performance achievable with mycobacterial automated culture systems in high and low prevalence settings. BMC
CONFLICT OF INTEREST
Authors declare no conflict of interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS’ CONTRIBUTION
Conception and Design: NHS, SB, FH, SBA, HU
Data collection, analysis & interpretation: NHS, SB, FH, NR
Manuscript writing: FH, NR, HU, SBA, AAZ