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Role of Gene Xpert Mtb/Rif in Diagnosis of Tuberculous Pus

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Abstract

Background- Extra-pulmonary tuberculosis (EPTB) remains a crucial challenge for the physicians with respect to timely diagnosis and treatment. The most common appearance of EPTB is tuberculous lymphadenitis with cervical lymphadenopathy being the most frequent. Many EPTB patients present with pus and discharging sinuses from lymph nodes, bones and joints.

Objective: To evaluate the performance of GeneXpert for detection of mycobacterium tuberculosis in clinically suspected cases of pus samples.

Methods: This prospective study was carried out in Dept. of Pulmonary Medicine, Govt. Medical College, Amritsar, from July 2015 to July 2017 and comprised pus samples of people having lymph node discharging sinuses, empyema, psoas abscess etc. The pus samples were subjected to Ziehl-Neelsen smear microscopy as per World Health Organisation's protocol and GeneXpert as per manufacturer protocol. Role of GeneXpert and Rifampicin resistance were determined in tuberculous pus samples.

Results: Out of the 142 pus samples, 85 (59.8%) were positive on GeneXpert and 38 (26.7%) on Ziehl-Neelsen smear. Rifampicin resistance was detected in 5 (5.8%) pus samples by GeneXpert.

Conclusion: GeneXpert MTB/RIF assay appears to be an accurate tool for rapid detection of MTB in pus samples. It has a good potential for diagnosis of extrapulmonary tuberculosis in resource-limited settings resulting in an improvement in patients' management and outcome.

Keywords: Gene Xpert MTB/RIF, Tuberculous Pus, EPTB, Rifampicin resistance.

Introduction

Tuberculosis (TB) is a global health concern. South East Asia carries about 40% of the global TB burden. India is highest TB burden country in the world and accounts for one fourth of the global TB burden cases. In 2015, an estimated 28 lakh cases occurred and 4.8 lakh people died due to TB.¹

Tuberculosis can involve any organ system in the body. While pulmonary tuberculosis is the most common presentation, extra pulmonary tuberculosis (EPTB) is also an important clinical problem.²⁻⁴ The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung. However, when an extra pulmonary focus evident in a patient with pulmonary tuberculosis, such patients have been categorized under pulmonary tuberculosis as per the guidelines of the World Health Organization (WHO).⁵

Extra pulmonary Tuberculosis (EPTB) accounts for about 15 to 20% of all cases of Tuberculosis in India.⁶ It can affect any organ of the body, most common type is lymph node TB and then pleural effusion, tubercular meningitis, abdominal TB, bone TB are other form of EPTB. The percentage may be higher in children and in HIV infected individuals. In HIV positive patients, EPTB accounts for more than 50 per cent of all cases of TB.⁷

Extra pulmonary infections with members of the Mycobacterium tuberculosis complex (MTBC) have high morbidity and mortality because of lack of good diagnostic methods. Diagnosis is often difficult to establish due to low number of bacteria and collection of extra pulmonary samples is not easy. A definitive diagnosis of mycobacterium infection depends on detection of the Mycobacterium Tuberculosis in extra pulmonary samples.⁸

The Gene Xpert MTB/RIF assay detects DNA sequences specific for Mycobacterium tuberculosis and Rifampicin resistance by polymerase chain reaction (PCR). It is also known as CBNAAT that is cartridge-based nucleic acid

amplification test having fully integrated and automated amplification and detection using real time polymerase chain reaction, providing result within 2 hours, It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target rpoB gene of MTB, no cross-reaction have been observed with other bacterial species tested, thereby excluding non-tubercular mycobacterium (NTM).⁹

Materials and Methods

This study was conducted in the Department of Pulmonary Medicine, Government Medical College, Amritsar. The study included 132 pus samples of patients with signs and symptoms suggestive of extra-pulmonary tuberculosis. This study was conducted after approval from the Institutional Ethics Committee and informed consent of each patient.

Inclusion criteria:

1. Patients having clinical feature of extra pulmonary tuberculosis.
2. Patients age more than 12 year of age.
3. Patients giving consent for the study.

Exclusion criteria:

1. Patients with features suggestive of pyogenic pus.
2. Patients on antitubercular treatment for more than 1 month.
3. Patients age less than 12 year of age.
4. Patients not giving consent for the study.

Complete history including past history, family history and history of contact of tuberculosis was taken. Complete physical examination was done. Aspiration of pus samples was done from various sites of body (cold abscess, empyema, lymph node and psoas abscess), and sent for ZN smear microscopy for AFB and Gene Xpert. Other investigation like TLC, DLC, Mantoux test, HIV etc were done. Chest x-ray and CT scan were done whenever needed.

Specimens were collected & transported whenever possible at 2 to 8°C in pre-sterilized falcon tubes and if sufficient volume of specimen is available, concentration methods used to increase yield by centrifugation. In lymph node and other tissue, 2ml of sterile phosphate buffer solution (PBS) is added, from this 0.7 ml of

homogenised tissue specimen is collected and mixed with double volume of Gene Xpert sample reagent (1.4ml). The processed sample is loaded into Gene Xpert MTB /RIF cartridge. The automated generated result was available in about 100 minutes.

Results

Table 1 Study Patients (Gender):

TOTAL	MALE	FEMALE
142	86(60%)	56(40%)

Out of 142 patients males were (60%), while females contributes (40%) of patients. The maximum number of patients belonged to the age group of 21-40 years and minimum patients were

in age group > 60 years. Mean age was 35.19 years. The youngest patient was 13 year old and the oldest patient was 77 year in age.

Table 2 Showing Correlation Between Pus For Afb By Zn Smear And Pus For Genexpert Assay

VARIABLE	PUS FOR GENEXPERT		
	POSITIVE	NEGATIVE	
Pus for AFB By ZN smear			$\chi^2 = 0.08,$ $df = 1,$ $p > 0.05$
NEGATIVE	47(45.2%)	57 (54.8%)	
POSITIVE	38(100%)	-	

Out of 104 pus samples which were negative on ZN smear microscopy, 47 were positive by Gene Xpert. Where all 38 samples which were positive

on ZN smear microscopy were also positive by Gene Xpert. However there was no statistically significant correlation between the two ($p > 0.05$).

Table 3 Gene Xpert MTB / RIF

TOTAL	Gene Xpert MTB / RIF		Rifampicin resistance
	POSITIVE	NEGATIVE	
142	85(59.8%)	57(40.2%)	5(5.8%)

The above table shows that out of 142 patients, 85(59.8%) were positive in Gene Xpert assay and 57(40.2%) were negative. Out of 85 Gene Xpert positive samples, 5(5.8%) were Rifampicin resistance.

samples obtained from relatively inaccessible sites being paucibacillary; all these factors decreasing the sensitivity of diagnostic tests. Since the conventional smear microscopy has a low sensitivity with a range of 0%–40%, negative results cannot exclude the presence of TB.¹⁰ The reported yields of mycobacterium culture vary from 30% to 80%, but it usually takes 2–8 weeks to receive the results, which is too slow to help treatment decisions.¹⁰ All these factors lead to a delay in diagnosis. Accurate and rapid laboratory investigations have therefore gained importance.

Discussion

Diagnosing EPTB remains challenging because of various reasons: symptoms varying depending on the organ involved, clinicians having low level of suspicion because of varied presentations, clinical

The present study was conducted on 142 clinioradiologically suspected patients of EPTB. The mean age in this study was 35.19 years. Higher prevalence of EPTB was noted in younger age which is similar to that reported by Arora VK, Gupta R.¹¹

In present study, out of total 142 pus samples 38 (26.7%) were AFB positive by ZN smear microscopy and 85 (59.8%) samples were positive by Gene Xpert Mtb/Rif. Out of these 85 positive samples 5 (5.8%) were Rifampicin resistance.

These findings of our study were similar to the study conducted by Srwar A et al¹² in which the yield of Gene Xpert was 31 out of 60 (51.7%) pus samples.

Similar study was also done by Shakeel K et al¹³ in which out of 212 pus samples, 77 (36.3%) were positive on Gene Xpert. Rifampicin resistance was detected in 5(6.4%) pus samples by Gene Xpert.

An overall sensitivity of 83.1% and a pooled specificity of 98.7% for diagnosis of EPTB was recently published in a meta-analysis by Denkinger et al.¹⁴ Raquel Moure et al¹⁵ in 2012 conducted a study; in which 58.3% were positive with the Xpert MTB/RIF assay for *Mycobacterium tuberculosis*. In a similar study by Vadwai V et al¹⁶ in 2011, the sensitivity of the Xpert assay was 81% (228/283 specimens), 64% for smear-negative cases and 96% for smear-positive cases), with a specificity of 99.6%.

The higher detection rate in above mentioned studies was due to the fact that they included diagnosed cases of TB (culture positive, smear positive) while our study was performed on suspected cases of EPTB.

Our study also justifies the INDEX TB guidelines 2017¹⁷ that have strongly recommended the use of Gene Xpert in pus samples.

Conclusion

Gene Xpert MTB/RIF assay is efficient and reliable technique for the rapid diagnosis of extra pulmonary TB, especially in suspected cases. Its simplicity, speed and automation, make this technique a very attractive tool for diagnosis of

Mycobacterium tuberculosis from extra pulmonary samples of TB suspects. Our findings suggest that Gene Xpert may have a role in EPTB diagnosis in addition to PTB, particularly in low-income/high-burden settings, where facilities for mycobacterial culture are limited.

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References

1. Annual Status Report. Revised National Tuberculosis Control Program, Chapter 2, TB Disease Burden in India. TB India 2017. Available in: <https://tbcindia.gov.in/WriteReadData/TB%20India%202017.pdf>
2. Fanning A. Tuberculosis: 6. Extrapulmonary disease. CMAJ: Canadian Medical Association Journal. 1999 Jun 1;160(11):1597. Available in: <http://www.cmaj.ca/content/160/11/1597.full.pdf>
3. Iseman MD. Tuberculosis in relation to human immunodeficiency virus and acquired immunodeficiency syndrome. In: Iseman MD, editor. A clinician's guide to tuberculosis. Philadelphia: Lippincott Williams and Wilkins; 2000, p. 199-252.
4. Dutt AK, Stead WW. Epidemiology. In: Schlossberg D, editor. Tuberculosis and nontuberculous mycobacterial infection. Philadelphia: W.B. Saunders Company; 1999, p. 3-16.
5. Maher D, Chaulet P, Spinaci S, Harries A. Treatment of tuberculosis: guidelines for national programmes. 1997. WHO, Geneva (WHO/TB/97.220).
6. Raj A, Singh N, Mehta PK. Gene Xpert MTB/RIF assay: a new hope for extrapulmonary tuberculosis. IOSR J Pharm. 2012 Jan;2(1):083-9.
7. Raviglione MC, Narain JP, Kochi A. HIV-associated tuberculosis in developing countries: clinical features, diagnosis, and treatment. Bulletin of the World Health Organization. 1992;70(4):515.

8. Hillemann D, Rüscher-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *Journal of clinical microbiology*. 2011;49(4):1202-5.
9. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A. Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine*. 2010;363(11):1005-15.
10. World Health Organization. Policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and Rifampicin resistance: Xpert MT. 2011. Available in: http://apps.who.int/iris/bitstream/10665/44586/1/9789241501545_eng.pdf
11. Arora V K, Gupta R. Trends of Extrapulmonary Tuberculosis under Revised National Tuberculosis Control Programme: A study from south Delhi. *Indian Journal of Tuberculosis*. 2006 Apr; 53(2): 77-83.
12. Srwar A, Akhtar R, Ahmad I, Mukhtar MN, Imran S, Akbar H, Ali S, Usman M. Rapid detection of Mycobacterium tuberculosis and Rifampicin Resistance in extra pulmonary samples using Gene Xpert MTB/RIF assay. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)* 2014 Nov; 13(11):50-53.
13. Shakeel K, Iram S, Akhtar M, Hussain S, Maryam H, Anwar A. Diagnostic validation of rapid molecular detection of Mycobacterium tuberculosis in pus samples by GeneXpert®. *J Pak Med Assoc*. 2018 Jan; 68(1):33-37.
14. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. *European Respiratory Journal*. 2014 Aug 1;44(2):435-46.
15. Moure R, Martín R, Alcaide F. Effectiveness of an integrated real-time PCR method for detection of the Mycobacterium tuberculosis complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. *Journal of clinical microbiology*. 2012 Feb 1;50(2):513-5.
16. Vadwai V, Boehme CC, Nabeta P. A new pillar in diagnosis of extrapulmonary tuberculosis? *Clin Microbiol*. 2011; 49: 2540-5.
17. Sharma SK, Ryan H, Khaparde S, Sachdeva KS, Singh AD, Mohan A, Sarin R, Paramasivan CN, Kumar P, Nischal N, Khatiwada S. Index-TB Guidelines: Guidelines on extrapulmonary tuberculosis for India. *The Indian Journal of Medical Research*. 2017 Apr;145(4):448.

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