International Journal of Research and Review
Vol.7; Issue: 10; October 2020
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Evaluation of Extra Pulmonary Tuberculosis in a Tertiary Care Centre in Kerala, South India, Utilising GeneXpert

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ABSTRACT

Even as pulmonary tuberculosis presents significant diagnostic challenges, diagnosis of extrapulmonary tuberculosis can be even more challenging. 1145 extrapulmonary samples were tested over a period of 2years, out of which Mycobacterium tuberculosis was detected in 144samples (12.6%). Samples included pleural fluid, lymph node aspirate & biopsy, cerebrospinal fluid and other sterile body fluids, synovial biopsy, ileocaecal biopsy and pus aspirates from abscess. Rifampicin resistance was detected in 6 samples (5 lymph node aspirates and 1 pleural fluid). All 6 were first line treatment failure cases who were started on second line antituberculous treatment. Xpert® MTB/RIF assay was found to be highly useful for diagnosis of lymph node tuberculosis and least for diagnosing tuberculous effusions.

Keywords **–** Tuberculosis, Extrapulmonary tuberculosis, GeneXpert, Rifampicin resistance, CBNAAT

INTRODUCTION

According to the World Health Organization Global Tuberculosis Report from 2019, an estimated10 million people fell ill with tuberculosis (TB) disease in 2018. Two third of the cases were in eight countries with India topping the list. [1]

Extrapulmonary tuberculosis (EPTB) represented 15% of the 7.2 million cases that were notified in 2018 worldwide. According to India TB report 2019, the incidence of TB in India is estimated to be 2.7 million. In Kerala, 24557 cases were notified in 2018 of which7523 were extrapulmonary (30%). [2]

EPTB can occur at almost any site, the most common being lymph nodes, pleura, genitourinary tract, bones and joints, meninges, peritoneum and pericardium in decreasing order of frequency. [3] Diagnosis of EPTB is difficult because of varied clinical symptoms and paucibacillary nature of the disease.

New methods that can aid in rapid and early diagnosis of TB are essential for initiating prompt treatment and thereby curtail spread of the disease. As an initiative for promoting affordable and quality TB diagnosis, WHO has endorsed a cartridge based nucleic acid amplification test (CBNAAT), the Xpert® MTB/RIF assay run on the GeneXpert platform (Cepheid Inc. USA), for diagnosis of TB.

We share our experience with Xpert® MTB/RIF assay for diagnosing EPTB from a tertiary care centre in North Kerala.

MATERIALS AND METHODS

Ethics – The study was carried out from June 2016 to May 2018. Approval has been obtained from Institutional Research committee and Institutional ethics committee.

Study design - cross sectional descriptive

All pulmonary and extrapulmonary samples received for CBNAAT during the

study period were followed up. Samples from patients with HIV infection have been excluded.

Only samples with adequate quantity were processed. Specimens were processed as soon as possible and within 48 hours. Specimens which could not be processed on same day were stored at 2-8°C.

All samples received in the laboratory for CBNAAT were processed in a BSL 3 laboratory according to the Xpert® MTB/RIF assay implementation manual by WHO. [4] The processed samples were inoculated into the cartridge and loaded in the GeneXpert (Cepheid) machine. The test utilizes molecular beacon to detect DNA sequences amplified in a hemi nested RT PCR. 5 different NA hybridization probes are used (A-E) in the same multiplex reaction which binds to wild-type, but not mutant target. The results are obtained within 2 hours as either of the following –

Mycobacterium tuberculosis not detected
Mycobacterium tuberculosis detected high/medium/low/very low load
Rifampicin resistance detected/not detected

Statistical methods

Results were analysed using Excel spreadsheet. The trend of extrapulmonary TB across various age groups was analysed in comparison with pulmonary TB cases diagnosed in the same period using chi square test.

RESULT

A total of 1843 pulmonary and 1145extrapulmonarysamples were processed during the study period.

No. of pulmonary tuberculosis diagnosed by CBNAAT – 669

No. of extrapulmonary tuberculosis diagnosed by CBNAAT – 144

Table 1 - Age wise distribution of disease

Age group (in years)	Extra pulmonary TB	Pulmonary TB	Proportion of EPTB
0-14	13/96	11/177	54.2
15-24	24/111	57/113	29.6
25-44	58/347	166/415	25.9
45-64	38/395	335/857	10.2
65+	10/196	100/281	9.1

EPTB showed a decreasing trend as age advanced, constituting only 9.1% of the total cases detected in the 65+ age group compared to 54.2% in the paediatric age group. χ^2 for trend = 50.9 (P value <0.001)

Table 2 –Site of involvement in different age groups

Age group	TB Lymph node	Skeletal TB	Cutaneous TB	Gastrointestinal TB	TB Meningitis	Pleural TB
0-14	8	3	0	1	1	0
15-24	16	4	1	1	0	2
25-44	24	14	0	5	3	12
45-64	14	10	0	1	3	11
>65	2	2	1	1	0	4

The various extrapulmonary samples processed and the positivity rate is given in the table below.

Specimen	Number of samples tested	Positive	%
Lymph node biopsy	18	12	66.7
Lymph node aspirate	118	52	44.1
Cold abscess aspirate	56	22	39.3
Peritoneal biopsy	3	1	33.3
Synovial biopsy	23	3	13.0
Bone biopsy	16	2	12.5
Ileocaecal biopsy	65	6	9.2
Serous cavity fluids	616	37	6.0
Skin biopsy	40	2	5.0
CSF	190	7	3.7
Total number	1145	144	12.6

The most common site of a tuberculous cold abscess was the paraspinal region (68%, n=15). Other sites included the chest wall, retroperitoneal cavity, anterior abdominal wall and anterior thigh. A case of TB osteomyelitis of the scapula also presented as an abscess on the back.

Table 3 - Positivity in serous cavity fluids

Specimen	Number of samples tested	Positive	%
Synovial fluid	63	6	9.5
Pleural fluid	444	29	6.5
Ascitic fluid	86	2	2.3
Pericardial fluid	23	0	0.0

Rifampicin resistance detected in pulmonary TB - 28 /669 (4.2%)
Rifampicin resistance detected in extrapulmonary TB - 6/144 (4.2%) -5 lymph node aspirate samples and 1 pleural fluid.

An indeterminate result for Rifampicin sensitivity was obtained in 6 extrapulmonary samples due to very low bacterial load - two synovial biopsy samples and one ascitic fluid, pleural fluid, ileocaecal tissue and lymph node tissue each. Sensitivity for these samples can be assessed only after obtaining growth in culture.

DISCUSSION

EPTB is a major public health issue, especially in countries like India with high TB burden. The actual prevalence of TB in Kerala remains unknown. So also the prevalence of extra pulmonary TB. 15-20 per cent of all cases of tuberculosis among immune-competent individuals are extrapulmonary and in HIV-infected patients it can go upto to 50 per cent. [1,5]
The varied clinical presentations, paucibacillary nature and the difficulty in obtaining specimens of adequate quantity and quality make the diagnosis of EPTB very difficult. Xpert® MTB/RIF is a semi-quantitative hemi-nested rt-PCR assay using molecular beacons. [6] It allows for rapid detection of MTB DNA along with rpoB gene mutation if present. Conventional methods of culture and drug sensitivity testing is the gold standard for diagnosis of TB. But this is a relatively complex and slow procedure requiring stringent biosafety precautions and trained laboratory personnel. Solid and liquid cultures require 4-8 weeks and 2-4weeks incubation respectively, making them prone to contamination. The Xpert® MTB/RIF assay system needs minimal technical skills and biosafety measures making it ideal for use in the periphery as a point-of-care test. It can diagnose TB within 2hours and is safe for handling after testing as the reagent used contains a tuberculocidal agent.

In our study, extrapulmonary TB constituted 17.7% of total TB cases detected during the study period. In young adults aged 25-44years, 25.9% of total TB cases were EPTB and in old age, 9.1%. But in pediatric age group, extrapulmonary TB was found to be more prevalent than pulmonary with 13 cases of extrapulmonary TB and 11 cases of pulmonary TB being detected during the study period (54.2%). There are very few studies from India on the prevalence of extrapulmonary TB in children. A study from Hubli, Karnataka has also reported EPTB to be more common in pediatric population (62.4%). [7] Two studies from Kerala on pediatric tuberculosis have reported 22% of cases to be EPTB. [8,9] A study conducted in National Institute of TB & Respiratory Diseases, New Delhi in 2019 had found only 8.1% of TB cases in children to be EPTB. [10] Though some studies have reported the incidence of EPTB to be more in adults, [11,12] several studies across the world have reported a similar finding of EPTB being more common in children <15years. [13-15]

There are studies which suggest that the proportion of extra-pulmonary TB in children could be used as an indicator of case detection in the region. [16] Estimating the burden of TB in children is very challenging. It is difficult to obtain expectorated sputum in children <5years. Gastric aspirate specimens are more difficult to obtain and only 20-50% are positive by either smear or culture. [16] Hence, a higher proportion of extra-pulmonary TB in children may also be due to cases of childhood pulmonary TB going undiagnosed.

The study was conducted in a referral centre and hence cases of childhood EPTB, which is more likely to be a diagnostic challenge compared to pulmonary TB, would be referred here, raising the proportion of EPTB.

In old age, pleural TB was the commonest form of EPTB (40%). In all other age groups, the most common lesion was tuberculous lymphadenitis (46.3%).

Similar findings were also observed in studies by Prakasha et al in Karnataka and Rai et al in Bihar. [11,17]

WHO has updated the overall sensitivity & specificity of different extrapulmonary samples by Xpert® MTB/RIF assay compared against a composite reference standard (CRS) based on 27 studies. [18]

Sample	sensitivity	specificity
CSF	55.5	98.8%
LN	83.7	99.2
PF	17	99.9
Tissue samples	81.2	98.1

In our study, Lymph node biopsy samples had the highest positivity (66.7%) followed by paraspinal abscess aspirate (15out of 23 samples tested positive for MTB - 65.2%). Lymph node aspirate had a positivity rate of 44.1%. Lymph nodes have been shown to be niches of mycobacterial growth and persistence in a study done done on macaques, and hence the high sensitivity. [19] CSF samples had the least positivity in our study (3.7%). Such low positivity rate for CSF samples from patients with suspected TB meningitis points at the need for ruling out alternate causes of chronic meningitis.

Serous cavity fluids also had a very low positivity rate with pericardial and ascitic fluids being the least. Low sensitivity of pleural and ascitic fluids in GeneXpert have been documented previously also. [20] There are very few studies which have sufficiently assessed the diagnostic accuracy of GeneXpert for pericardial and synovial fluids. Hence, this platform is not recommended for diagnosis of tuberculous effusions now.

There are no accurate statistics on prevalence of drug resistance in EPTB, especially in India. Incidence of drug resistant tuberculosis (DRTB) in Kerala in 2018 was about 1% (pulmonary and extrapulmonary included). This is lower than the neighbouring states of Tamilnadu, Karnataka and Andhra Pradesh. [2] In our study, rifampicin resistance was seen in 4.2% of extrapulmonary as well as pulmonary TB cases, which is higher compared to the state data. This could be due to referral of treatment failure cases to our institution which is a DRTB nodal centre. A study from IRL Kerala in 2017 estimates the positive predictive value of Xpert for rifampicin resistance as only 79% by rechecking the results of Xpert with phenotypic method using MGIT. [21] A Cochrane Review which assessed the accuracy of Xpert in extrapulmonary specimens found the sensitivity (20 studies, 148 specimens) and specificity (39 studies, 1088 specimens) to be 95.0% and 98.7%, respectively. [22] In our study, all the six cases of rifampicin resistance were from patients who had not responded to first line ATT, and all the patients were initiated on Category IV drugs based on the Xpert MTB/Rif report.

CONCLUSION

Extrapulmonary tuberculosis cases are on the rise in the region especially in the pediatric population. Lymph node tuberculosis accounts for maximum number of cases, but pleural effusion is commoner as age advances. Xpert® MTB/RIF assay is a very reliable technique for rapid detection of tuberculosis, especially extrapulmonary tuberculosis, as cultures often get contaminated during prolonged incubation. It is highly useful for diagnosis of lymph node tuberculosis and least for diagnosing tuberculous effusions. Simultaneous detection of rifampicin resistance helps in timely initiation of appropriate therapy, significantly improving tuberculosis care.

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How to cite this article: Jose BP, Kumar AP, Mohan A. Evaluation of extra pulmonary tuberculosis in a tertiary care centre in Kerala, South India, utilising GeneXpert. International Journal of Research and Review. 2020; 7(10): 551-556.

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