

Research Article

Study of Extra Pulmonary Tuberculosis using Gene X-pert and Fluorescent Microscopy at Vasant Rao Naik GMC Yavatmal

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ABSTRACT

Background: Tuberculosis remains a major global health problem with 1/3 of world's population being infected with tuberculosis. Along with HIV, Diabetes mellitus and recently evolved drug resistant tuberculosis, it is a big threat for mankind. TB can involve any organ system in the body. While PTB is the most common presentation, EPTB is also an important clinical problem.

Aim and Objectives: 1. Study of Extra Pulmonary Tuberculosis using Gene X-pert and Fluorescent Microscopy at Vasant Rao Naik GMC Yavatmal. 2. Study the age and gender wise distribution of Extra pulmonary tuberculosis patient. 3. Finding associated risk factors with extra pulmonary tuberculosis.

Methods: **Study Design:** Cross sectional study. **Study Population:** All extra pulmonary suspected patients admitted in Vasant Rao Naik GMC Yavatmal during study period such cases were included in the study. **Study Duration:** 2023-2025 **Sample size:** 246

Results: Most of the EPTB cases found in 26-40 years age group 87 (36.25%) followed by 62 cases (25.83%) in 41-55 years age group, 39 (16.25%) in 12-25 age group, 37 (15.41%) in 56-65 age group and 21 cases found in above 66 years age group. Majority of EPTB cases were males 149 (60.56%) and females were 97 (39.44%). EPTB cases as per diagnosis majority of cases were males 149 and 97 cases were females. In males most of cases diagnosed pleural TB 69 (46.30%) followed by lymph node TB 32 (21.47%), abdominal TB 27 (18.12%), Pott's spine 12 (8.05%), TB meningitis 4 (2.68%), peritoneal TB 4 (2.68%) and genital TB 1 (0.67%). Association of Pleural TB cases with age was statistically significant at $p < .05$. Sensitivity and specificity of Gene X-pert and Fluorescent Sensitivity=89.43%, Specificity= 70.58%, Positive Predictive value = 94.82% and Negative Predictive value = 16.12%

Conclusions: Most of the EPTB cases found in 26-40 years age group, majority of EPTB cases were males, In males most of cases diagnosed pleural TB, Association of Pleural TB cases with age was statistically significant at $p < .05$. Sensitivity and specificity of Gene X-pert and Fluorescent Sensitivity=89.43%, Specificity= 70.58%, Positive Predictive value = 94.82% and Negative Predictive value = 16.12%

Keywords: EPTB, Gene X-Pert and Fluorescent, Pleural TB, Lymph Node TB, Abdominal TB, Pott's Spine.

INTRODUCTION

Tuberculosis remains a major global health problem with 1/3 of world's population being infected with tuberculosis. Along with HIV, Diabetes mellitus and recently evolved drug resistant tuberculosis, it is a big threat for mankind. TB can involve any organ system in the body. While PTB is the most common presentation, EPTB is also an important clinical problem. [1-4]

The term EPTB has been used to describe isolated occurrence of TB at body sites other than the lungs. Diagnosis of EPTB is done as per RNTCP guidelines which is based on one culture positive specimen from extrapulmonary sites or histological evidence or strong clinical evidence consistent with active EPTB followed by medical officer's decision to treat with a full course of anti tubercular therapy under DOTS [5,6]. Atypical presentation, lack of diagnostic resources for procurement of tissue or fluid for diagnosis from an accessible sites and a poor yield of conventional diagnostic methods leads a considerable delay in making the diagnosis or diagnosis may be missed. [5]

Lately due to the availability of sophisticated investigations like CT, endoscopy, MRI, have tremendously helped in localisation of anatomical site for obtaining tissue for diagnosis. The percentage of EPTB among all TB cases in developed countries ranges from 12 to 28.5%. In developing countries like India, the percentage of EPTB cases is between 15- 20% which has

increased to more than 50% among HIV co infected patients [7,8] suggesting immunity status of host being a major risk factor of EPTB.

EPTB constitutes about 15-20% of all cases of tuberculosis in immunocompetent individuals.[7] But in immunocompromised individuals like HIV, diabetes mellitus, COPD, cancers, malnutrition, chronic renal diseases, liver diseases, post organ transplant etc its incidence rises.[7,8] In HIV positive TB patients, EPTB accounts for more than 50%(1). Globally lymphatics and lymph node TB is the most common form of EPTB [10-12] found in studies from Ethiopia, Canada, Turkey, Nepal and other Indian studies.[13-16] The order of occurrence of EPTB is similar to RNTCP reports.[17]

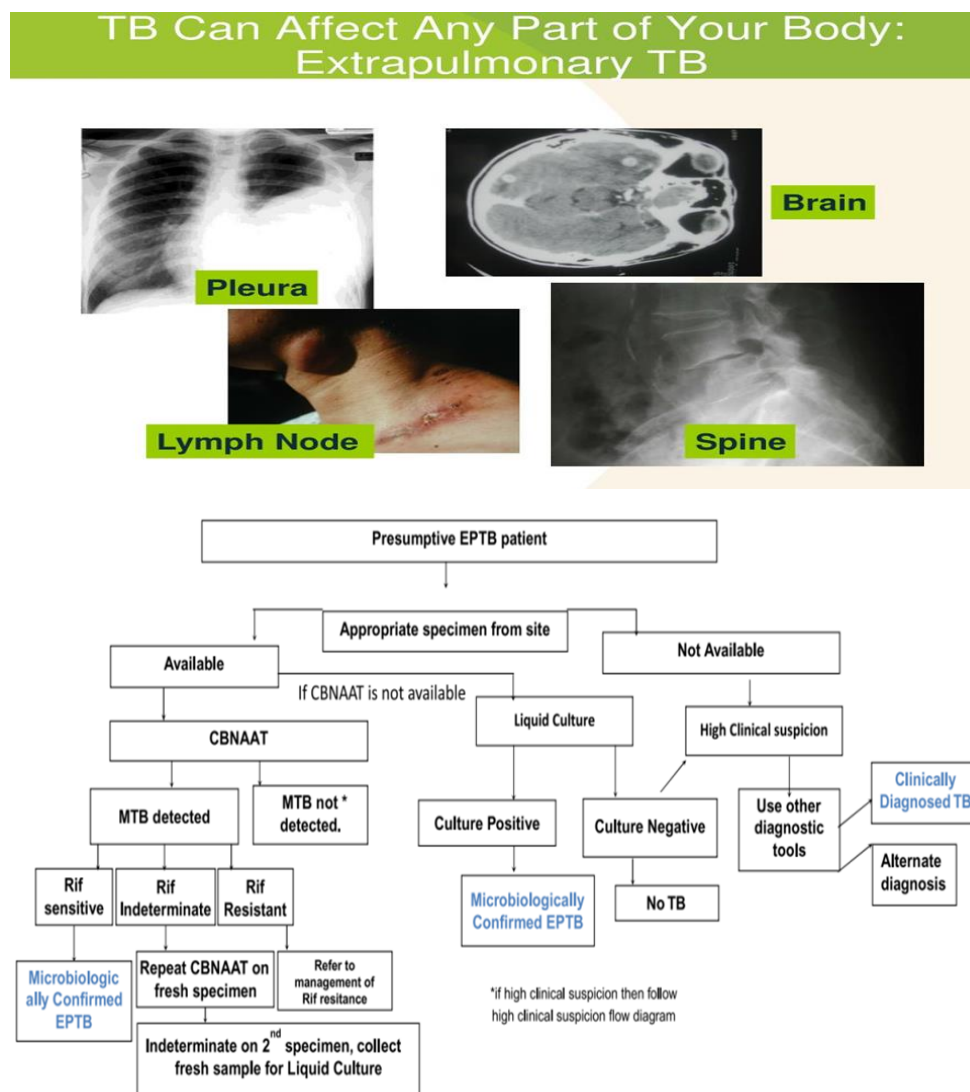


Figure no 2: Diagnostic Algorithm of EPTB

AIM AND OBJECTIVES

Aim: Study of Extra Pulmonary Tuberculosis using Gene X-pert and Fluorescent Microscopy at Vasantnao Naik GMC Yavatmal

Objective

1. Study the age and gender wise distribution of Extra pulmonary tuberculosis patient
2. Finding associated risk factors with extra pulmonary tuberculosis

MATERIAL AND METHODS

Study design: Cross sectional study

Study setting: Department of Microbiology, Vasantnao Naik GMC Yavatmal

Study Population: All extra pulmonary suspected patients admitted in Vasantnao Naik GMC Yavatmal during study period such cases were included in the study

Type of sampling: Convenient sampling technique

Duration of study: 18 months

Sample size: 246

Sample size calculation: The sample size was calculated by formula of estimating proportion formula Yang et al study in patients with extra-pulmonary tuberculosis

Sample size= $Z^2 \times (p-q)/d^2$ $Z=1.96$ $P=80\%=0.8$ $Q=1-P=0.05$

$N=(1.96)^2 \times ((0.8 \times 0.2)/(0.05)^2)$

Total sample size= 246 patients

Inclusion Criteria

- Age > 12 years
- All extra pulmonary suspected patients admitted in ward of tertiary care hospital in central India
- Patient who gives consent.

Exclusion Criteria

- Age < 12 years
- All pulmonary cases.
- Pulmonary Tuberculosis with Extra pulmonary Tuberculosis.
- Patient who are not ready for consent.

A cross sectional study was conducted in Microbiology department of Vasantnao Naik GMC Yavatmal after ethics committee permission. Suspected EPTB patients who came to our general medicine OPD and IPD and who fulfilled the inclusion criteria were considered for the study. Informed consent is taken from all study participants.

Extra pulmonary cases included in our study were lymph node TB cases, TB pleural effusion cases, TB meningitis cases, Tuberculoma cases, TB spine cases, peritoneal TB cases, Abdominal TB cases, genital TB cases etc.

Demographic data, clinical history, examination findings and radiological tests of these patients were noted. Diagnosis of TB was done with the help of Gene X-pert and Fluorescent Microscopy. Patients who had no or incomplete data were excluded from the study.

USG guided lymph node biopsy and FNAC samples in lymph node TB cases, pleural fluid analysis and chest x-ray in TB pleural effusion cases, CSF studies and CNS imaging in TB meningitis cases, imaging in spinal TB cases, ascitic fluid studies and ultrasonography in abdominal TB cases and various other radiological and biochemical tests were collected and observed. Case proforma sheets are filled and the data is entered in MS excel sheet.

GeneXpert MTB/RIF assay and LED-FM microscopy:

Smear for LED fluorescent microscopy were prepared according to standard procedures. Smears for FM were stained using auramin O. Briefly, smears were flooded with Auramin O for 10 min, destained with acid alcohol for 2 min, and then counterstained with methylene blue for one minute. With auramine O staining, *Mycobacteria* appear as bright yellow fluorescent rods on a dark background.

The slides were examined with ZEISS EXTARO 300 at 20× magnification. The presence or absence of AFB was reported using WHO/IUATLD guideline. The sediment samples by cytocentrifugation were processed for Xpert MTB/RIF assay. Using a fresh transfer pipette, 2 mL of the processed sample was transferred to the Xpert MTB/RIF cartridge 10. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions according to standard protocol.

Statistical analysis:

Data entry done using M.S. Excel and it will be statistically analyzed using Statistical package for social sciences (SPSS Version 25) for M.S. Windows. Descriptive statistical analysis carried out to explore the distribution of several categorical and quantitative variables. Categorical variables summarized with n (%), while quantitative variables will be summarized by mean S.D. All results presented in tabular form and are also shown graphically using bar diagram or pie diagram among genders as appropriate. Inferential Statistics: categorical variables tested by Chi-Square test. P-value less than 0.05 considered to be statistically significant.

OBSERVATION AND RESULT

A cross sectional study was conducted in Microbiology department of Vasantao Naik GMC Yavatmal after ethics committee permission. Suspected EPTB patients who came to our general medicine OPD and IPD and who fulfilled the inclusion criteria were considered for the study.

Table No. 1: Distribution of Cases According to Age (N=246)

Age in years	Frequency	Percentage
12-25	39	16.25%
26-40	87	36.25%
41- 55	62	25.83%
56-65	37	15.41%
Above 66	21	8.75
Total	246	246 (100%)

The table shows most of the EPTB cases found in 26-40 years age group 87 (36.25%) followed by 62 cases (25.83%) in 41-55 years age group, 39 (16.25%) in 12-25 age group, 37 (15.41%) in 56-65 age group and 21 cases found in above 66 years age group

Table No.2: Distribution of EPTB Cases as Per Sex (N=246)

Gender	Frequency	Percentage
Male	149	60.56%
Female	97	39.44%
Total	246	246 (100%)

The above table shows majority of EPTB cases were males 149 (60.56%) and females were 97 (39.44%).

Table No.3: Distribution of EPTB cases as per Diagnosis (N=246)

Diagnosis	Male: N (%)	Female: N (%)
Pleural TB	69 (46.30%)	18 (18.55%)
Lymph node TB	32 (21.47%)	47(48.45%)
Abdominal TB	27 (18.12%)	14 (14.43%)
Potts spine	12 (8.05%)	2(2.06%)
Genital TB	1 (0.67%)	7(7.21%)
TB Meningitis	4 (2.68%)	6 (6.18%)
Peritoneal TB	4 (2.68%)	3 (3.09%)
Total	149 (100%)	97 (100%)

The above table shows EPTB cases as per diagnosis majority of cases were males 149 and 97 cases were females. In males most of cases diagnosed pleural TB 69 (46.30%) followed by lymph node TB 32 (21.47%), abdominal TB 27 (18.12%), potts spine 12 (8.05%), TB meningitis 4 (2.68%), peritoneal TB 4 (2.68%) and genital TB 1 (0.67%)

Table No. 4: Association of Pleural TB cases with age (N=246)

Age in years	Pleural TB	Frequency	P value
12-25	16	39	0.00001
26-40	41	87	
41- 55	10	62	
56-65	11	37	
Above 66	9	21	
Total	87	246	

The chi-square statistic is 20.2071 the p value is 0.00001. Significant at $p < .05$, Association of Pleural TB cases with age was statistically significant at $p < .05$.

Table no.5: Sensitivity and Specificity of GeneXpert

GeneXpert	fluorescent microscopy	
	Disease	Not Disease
Positive	220	12
Negative	26	5
Total	246	17

Sensitivity=89.43%, Specificity= 70.58%, Positive Predictive value = 94.82% and Negative Predictive value = 16.12%

DISCUSSION

A cross sectional study was conducted in Microbiology department of Vasantnao Naik GMC Yavatmal after ethics committee permission. Suspected EPTB patients who came to our general medicine OPD and IPD and who fulfilled the inclusion criteria were considered for the study.

In current study most of the EPTB cases found in 26-40 years age group 87 (36.25%) followed by 62 cases (25.83%) in 41-55 years age group, 39 (16.25%) in 12-25 age group, 37 (15.41%) in 56-65 age group and 21 cases found in above 66 years age group. Similar result found in the study conducted by Ashok Kumar Bhardwaj et al [83] He reported that the observed mean age was 36.9 and 33.6 year ($p=0.00$) for male and female respectively. And 61.5% of the patients were less than 35 years of age.

Shrivastava AK et al [18] He observed that the age group of 20-39 years had the highest proportion of EPTB both in males and females which is the economically productive population of society. Anita velingker, et al [19] he reported that the age group of 30- 50 years had highest proportion extra pulmonary tuberculosis both in males and females. Ravikumar P et al [20] He found that the Among 224 cases of extra-pulmonary TB studied, 136 (60.7%) were males and 88 (39.3%) were females and most of the patients were in the age group of 21-40 years.

In current study majority of EPTB cases were males 149 (60.56%) and females were 97 (39.44%). Anita velingker, et al [19] reported that the prevalence of extra pulmonary cases was higher among males as compared to females. Krossnnpunui et al [21] Out of 1502 patients, 769 (51.2%) were extra pulmonary tuberculosis. Higher number of cases- 54.3% (815) was male and 45.7% (687) were female. Ravikumar P et al [20] among 224 cases of extra-pulmonary TB studied, 136 (60.7%) were males and 88 (39.3%) were females.

In current study shows EPTB cases as per diagnosis majority of cases were males 149 and 97 cases were females. In males most of cases diagnosed pleural TB 69 (46.30%) followed by lymph node TB 32 (21.47%), abdominal TB 27 (18.12%), potts spine 12 (8.05%), TB meningitis 4 (2.68%), peritoneal TB 4 (2.68%) and genital TB 1 (0.67%). Shrivastava AK et al [18] among 491 cases registered for treatment of all forms of tuberculosis, 361(73.53%) had PTB and 130 (26.47%) had EPTB. The ratio of percentage EPTB: PTB is 1:3.6. Commonest type of EPTB was found in cases of lymph nodes and lymphatic (30.76%), followed by TB in pleural cavity (23.03%).

Heather M Peto et al [22] found that the Among 253,299 cases, 73.6% were PTB and 18.7% were EPTB, including lymphatic (40.4%), pleural (19.8%), bone and/or joint (11.3%), genitourinary (6.5%), meningeal (5.4%), peritoneal (4.9%), and unclassified EPTB (11.8%) cases. Ravikumar P et al [20] He revealed that the 224 cases of extra-pulmonary tuberculosis, most common site of extra-pulmonary tuberculosis was pleura (29.9%) followed by meninges (22.5%), abdomen (19.6%) and lymph node (10.7%) tuberculosis. The least common sites were hip joint, elbow joint, omental mass, skin TB and miliary TB.

In current study most of the PTB cases found in 26-40 years age group 41 followed by 16 cases in 12-25 age group, 11 cases in 56-65 age group, 10 in 41-55 age group and 9 cases in above 66 age group. Association of Pleural TB cases with age was statistically significant at $p<.05$. Heather M Peto et al [22] found that the Association of Pleural TB cases with age was statistically significant at $p<.05$. In current study Sensitivity and Specificity of GeneXpert was Sensitivity=89.43% and Specificity= 70.58%. Verma D et al [23] He found that the among these 35 GeneXpert cases 16 cases, were confirmed positive by fluorescent microscopy. Considering GeneXpert as a standard, fluorescent microscopy was found to be 45.7% (95% CI, 28.83% to 63.35%) sensitive and 100% specific (98.21% to 100.00%).

CONCLUSIONS

Most of the EPTB cases found in 26-40 years age group, majority of EPTB cases were males, In males most of cases diagnosed pleural TB, Association of Pleural TB cases with age was statistically significant at $p < .05$. Sensitivity and specificity of Gene X-pert and Fluorescent Sensitivity=89.43%, Specificity= 70.58%, Positive Predictive value = 94.82% and Negative Predictive value = 16.12%

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