

Original Research Article

## **Comparative Analysis of Gene Expert Assay In Addition To Z-N Microscopy for Detection of Extra -Pulmonary TB in A Fine Needle Aspiration Samples**

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**Abstract:** Tuberculosis (TB) remains one of the worlds deadliest communicable disease. Extrapulmonary tuberculosis (EPTB) can occur in isolation or along with pulmonary focus with disseminated tuberculosis. Since clinical presentation of EPTB is atypical and poor yeild of conventional diagnostic method, the diagnosis often delayed. Though Z-N staining low cost,high specificity and does not required high laboratory standards, the gene Xpert MTB/RIF assay provide early ,distinguishes MTB and mycobacteria other than tuberculosis and also provide rifampicin resistance simultaneously in a Fine needle aspirated sampling. The aim is to assess and to compare the value of GeneXpert in diagnosis of EPTB in addition Ziehl-Neelson smear microscopy in Fine needle aspirated samples. It was retrospective study carried out in department of laboratory medicine, Yashoda hospital, Malakpet branch Hyderabad during period of November 2015 to July 2017 (twenty months). Forty, immunocompetent, out patient cases from all departments, diagnosed as extrapulmonary tuberculosis evaluated with Z-N smear microcopy and GeneXpert in Fine needle aspiration sampling with or without radiological assistance. EPTB incidence was 3.5%. Females are more commonly affected with male to female ratio 1:2.Second to third decade was commonly affected group. Cervical lymphadenopathy was most common presentation (62.5%). Epitheloid granulomas with caseation necrosis (40%) was most common cytological picture. Overall AFB positivity seen in 62.5% compare to 100% positive gene X-pert findings and Rifampicin resistance seen in 2.5% cases. EPTB diagnosis is great challenge. Image guidance FNAC plays first line investigation. GeneX-pert has shown higher levels of sensitivity and specificity for diagnosis of EPTB compare to cytology and Z-N microscopy.

**Keywords:** EPTB; FNAC; Gene X-PERT,; Cytology ; Z-N staining.

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### **INTRODUCTION:**

India, the world's second most populous country, accounts for a quarter of the world's annual incidence of TB. Every year around two million people develop TB in India and 300,000 die of TB. Even though TB is a curable disease it is still manages to kill around 5000 people every day according to World Health Organization (WHO)[1]. The EPTB term has been used to describe isolated occurrence of tuberculosis at body sites other than the lung. However when extrapulmonary focus evident in a patient with pulmonary tuberculosis, such patient have been categorized under pulmonary tuberculosis as per guidelines of WHO organization. EPTB constitute

about 15-20%of all cases of tuberculosis in immunocompetent patients and accounts for more than 50%of cases in HIV positive individuals [2]. Extrapulmonary sites of infection commonly include lymph nodes, pleura, osteoarticular areas, gastrointestinal & genitourinary system, although any organ can be involved [3].

Image guided fine needle aspiration cytology (FNAC)/ biopsy has emerged as the first line of investigation in the assessment of radiologically detected lesions. This is a safe, less traumatic, rapid and easy method compared to larger core or open biopsy. This procedure is cost effective as well as easier to

repeat, if necessary [4]. Diagnosis of EPTB depends on detection and demonstration of mycobacterium and its products (Direct method) and the indirect method to diagnose EPTB depends on measurement of the host humoral and cellular response against mycobacterium.

Mycobacterium tuberculosis can be stained from aspirated samples by histological staining (Ziehl-Neelsen), which stains up bright red with blue background. This is method of choice because of low cost, high specificity and does not require sophisticated equipment. But this is less sensitive, no species, no viable bacteria & no drug susceptibility detected with this method. Positive Z-N smear requires more than 5000-10000 bacilli/ml, so of limited diagnostic value (0-40%) in majority of pauci-bacillary EPTB samples [5].

During the last decade, a number of nucleic acid amplification (NAA) methods have been developed for rapid detection and identification of *Mycobacterium tuberculosis* (MTB) in clinical specimens. The Xpert MTB/RIF assay is a semiquantitative nested real-time PCR for detection of Mycobacterium tuberculosis complex DNA and rifampicin-resistance associated mutations of rpoB gene in samples from patients at risk for rifampin resistance. WHO has strongly recommended this test for diagnosis of TB in various settings [6].

#### MATERIALS AND METHODS:

This is retrospective study was carried out at Yashoda Hospital, Malakpet, Hyderabad over a period of twenty months from November 2015 to July 2017. Out of 1146 total FNAC, 40 cases were selected for study. Patients age, gender, site of lesions, gross examination of aspirate, cytomorphological patterns, stain for AFB (acid fast bacillus) and result of GeneXpert/MTB assay results were studied. FNAC without guidance done in 28 cases & with radiological assistance (ultrasound guided 07 cases, CT scan guided 03 cases and Endoscopic ultrasound guided 2 cases). All cases were OP basis and FNAC done using 22 gauge needle with 5 and 10 cc syringe. Image Guided FNAC done with the help of Lumbar puncture needle no.20 (Black colour) and 18 Gauge (pink colour) needle for osteoarticular lesion. In each case minimum two pass or two attempts done from same lesion. First pass sample collected for cytology slides for which minimum three

slides, one for H&E stain (Fixative) two dry slides for Giemsa and Z-N stain respectively. Second pass sample collected in sterile vacutainer for Gene Xpert/RIF assay testing.

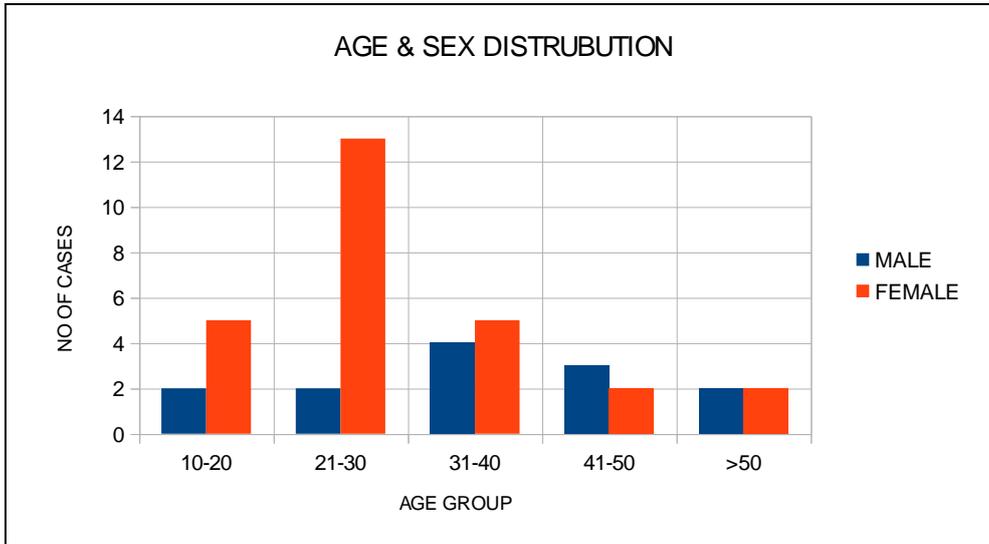
The cytology smears revealing features of tubercular lymphadenitis were categorised as per different morphological patterns i.e. Granulomas with caseous necrosis, Granulomas without necrosis, Caseous necrosis only and Suppurative inflammation. For each Z-N staining microscopy with known positive and negative slides were included. A minimum of 100 oil immersion fields were seen before reporting a smear as negative. A positive slide contained at least 3 acid fast bacilli among 100 oil fields of microscope. An experienced microbiologist rechecked the smears for internal quality.

The XpertMTB/RIF assay is a semiquantitative nested real time PCR was performed according to the manufacturer's instruction. A semi quantitative estimate of the concentration of bacilli can be defined on the cycle threshold (ct) value as ct value high <16; Medium:16-22; Low 22-28; very low >28.

The present study, detected mycobacterium tuberculosis from FNA assisted cases by Xpert MTB/RIF assay retrospectively compared with AFB microscopy and incidence, cytomorphology pattern with incidence of rifampicin resistance status in OP cases diagnosed as extrapulmonary TB were studied.

#### RESULTS:

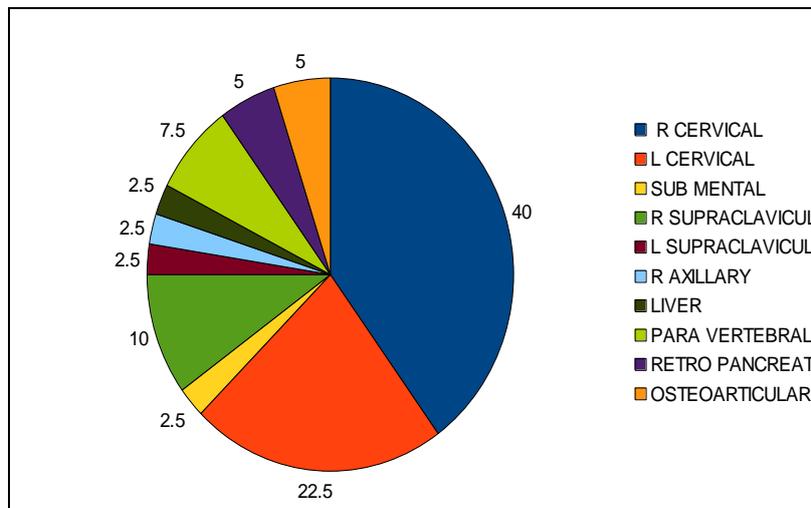
This study was carried out for 20 months in cytopathology and microbiology section, Department of laboratory medicine. Out of 1146 FNAC samples aspirated OPD bases, 40 cases were diagnosed as EPTB and incidence was reported 3.5% over all FNAC aspirated diagnosis. There were 13 males and 27 females. The mean age was 35 years with the range of 10 years to 80 years. Most common affected age group was 21 to 30 years (2 Male & 13, Females)(37.5%), 31-40 years (4 Males & 5 Females)(22.5%), 41-50 (3 Males & 2 Females)(12.5%) years, 10-20 years (2 Males & 5 Females), (17.5%) and least was more than 50 years (2 Males & 2 Females) (10%) age group (Chart 1). Female patients are predominant in almost all age groups except age group of 41-50 years were male patients predominate.



**Chart: 1; Age and Sex Distribution in Extra pulmonary TB cases.**

Around 62.5% of cases had cervical presentation(Right 40%> left 22.5%) followed by Right supraclavicular (10%), Paravertebral (7.5%), reteropancreatic and osteoarticular 5% each while

Submental, Left supraclavicular, Right axillary, & Liver share 2.5% each (diagram1). There was no case with generallised lymphadenopathy.



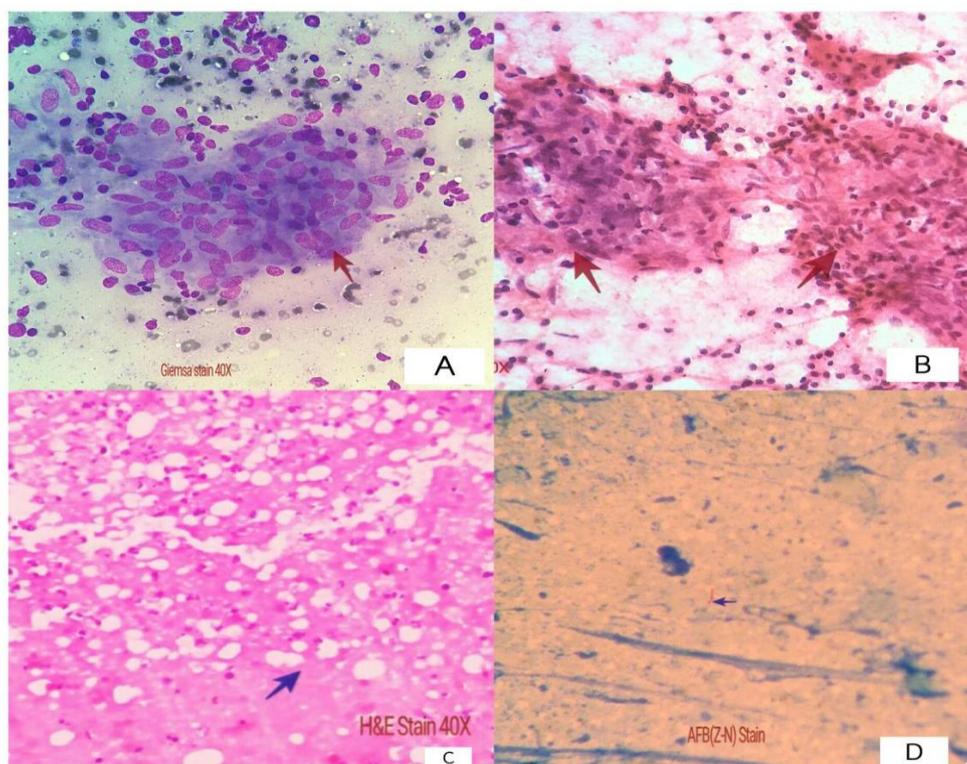
**Diagram: 1; Distribution of cases as per involved Anatomical sites**

Gross findings in aspirated samples predominantly show 55% of blood mixed purulent, followed by pus 30%, dry paste like or chessy material and hemorrhagic 7.5% each. Cytomorphology showed commonly Granuloma with caseous necrosis 16 cases(40%) , followed by Suppurative inflammation (27.5%), Granulomas without caseous necrosis(22.5%) and Caseous necrosis with nuclear debris(10%) cases.

Overall stain for AFB positive in 62.5% cases. The percentage of positivity of AFB staining (Z-N stain) most commonly seen with granulomas with caseous necrosis (37.5%) followed by suppurative inflammation(15%), Granulomas without caseous necrosis(10%) and 7.5% seen in caseous necrosis (Table 1).

**Table: 1; Cytomorphology patterns and AFB Positivity in different patterns**

Sr.No	Cyto-morphology of aspirated sample	No. of cases	Of AFB positive	% positive	AFB Negative	% Negative
1	Granulomas with Caseous necrosis	16(40%)	15	37.5%	01	2.5%
2	Granulomas without Caseous necrosis	09(22.5%)	04	10%	05	12.5%
3	Caseous necrosis & nuclear debris	04(10%)	03	7.5%	02	2.5%
4	Suppurative inflammation	11(27.5%)	06	15%	05	12.5%



**Figure1:1;A;B: show granulomas consisting epithelioid cells(Red arrow) C; Caseous necrosis(blur arrow) D;Z-N stain show positive AFB (blue arrow)**

Gene X-pert cycle threshold (ct) detected low in 23(57.5%) cases followed by 17(42.5%) And very low 01(2.5%).None of the case resulted with high density. So there are 15 ZN smear negative subjects (37.5%) were found to be positive for MTB by Gene X-pert with Rifampicin Resistance found in one case.

**DISCUSSION:**

The annual global incidence of EPTB has been increasing in the last decade due to the changing TB

control practices, spread of HIV and population growth. The diagnosis of EPTB poses particular challenge for clinicians because of its atypical presentations and lacking of health facilities in rural areas that are used by about 70% of the populations in developing countries like, India [7]. Diagnosis of these condition requires high clinical suspicion and special diagnostic procedures. A negative smear of Acid Fast Bacillus (AFB), Lack of granulomas in cyto-histopathology and failure to culture MTB do not

exclude diagnosis. Fine needle aspiration cytology (FNAC) with or without guidance is a simple out-patient diagnostic procedure used for the diagnosis of palpable and deeply seated lesions.

During last decade, a number of nucleic acid amplification (NAA) methods have been developed for rapid detection and identification of MTB [8]. Newly administered Gene Xpert MTB/RIF assay take two hours to provide the final results, in addition distinguishes MTB & mycobacteria other than tuberculosis and also provide rifampicin resistance simultaneously. Polymerase chain reaction (PCR) is based on NAA methods and is widely used for the rapid diagnosis of TB [9].

The present study aimed to find out AFB positivity compare to positive Gene Xpert detection of FNAC samples, also to study the incidence and cytomorphology in extrapulmonary tuberculosis diagnosed on OPD basis. In Our study, maximum no. of patients were present in 21-30 years, with mean age 27. Less cases are seen in extreme ages. Female patients are more commonly affected. Cervical lymph nodes commonly involved. Most common cytomorphology was epithelioid granulomas with caseous necrosis (40%). Similar findings were reported by Purohit *et al* [10], Gupta *et al* [11], Chand P [12] and Paliwal *et al* [13]. AFB smear positivity in present study was found to be 62.5% compare to all Gene Xpert positive cases. Comparable rates of AFB positivity were reported by Ergete *et al* [14]. Maximum smear positivity present in caseous necrosis with granulomas cases (45%).

Culture being gold standard but take 4-6 weeks for diagnosis. Newer Faster radiometric (BACTEC) and non-radiometric methods like fluorescent labeled mycobacterium growth indicator test (MGIT) methods being used and provide results in 7-10 days. It is time consuming and equipment expenses are much high, more skilled personals, continuous monitoring for several days and further confirmation of positive cultures by making ZN smears are required [15]. Efficacy of Lowenstein Jensen medium to detect MTB has been demonstrated as 10 viable bacilli per ml of specimen while PCR can detect as low as 10 fg of bacilli which is equal to 2 bacilli per ml. Although Rifampicin resistance was less (2.5%) in present study but detection helps in further management.

#### CONCLUSION:

To Conclude, Diagnosis of EPTB is difficult due to its atypical presentation and requires high clinical suspicion and special diagnostic procedures. FNAC with imaging proves as very useful first line investigation. Gene Xpert testing with rifampicin resistance test in aspirated samples has shown significantly higher levels of sensitivity and specificity for diagnosis of EPTB as compared to cytomorphology and Z-N staining microscopy alone. Also conclude that, combined techniques proved itself as an effective tool in OP basis diagnosis and early initiation of treatment to prevent morbidity, mortality and economic loss.

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