

The Role of Various Laboratory Techniques for Diagnosis of Palpable Extrapulmonary Tuberculosis - A District Hospital based Retrospective Study

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Abstract: ***Background:** EPTB is TB outside lung. The protean manifestation of EPTB can involve almost any system of the body. The study attempts to determine the prevalence of EPTB presenting as palpable lumps using combination of various diagnostic techniques. **Method:** Retrospective study was conducted. Data were collected from Pathology and Microbiology record books of North District Hospital. The analysis of all palpable EPTB diagnosed by FNAC from April 2016 to March 2021 was done. Demographic data included age, sex, site affected. Study of gross aspirate, cytomorphology with NAAT, AFB and culture was done. **Results:** 66.38% of PTB and 33.62% of EPTB were seen. The commonest site was cervical lymph node (67.2%). Women were more affected (51.4%) than men (48.5%). Cheesy and purulent aspirate showed higher percentage of AFB and NAAT positivity. The sensitivity of AFB (53.6%) and NAAT (76.2%). **Conclusion:** EPTB is a complex disease and needs an appropriate, early tissue based diagnosis adjuvant with other microbiological tests which are reliable, economical and can be used for rapid diagnosis. This will help in directed treatment and avoid developing resistance. High incidence of EPTB needs further study.*

Keywords: AFB, FNAC, NAAT, paucibacillary

1. Introduction

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis. It is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS) ¹. India accounts for the highest TB burden in the world (26%), having an estimated incidence of 26.9 lakh cases in 2019 ². While pulmonary TB is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical condition³. The burden of EPTB is high ranging from 15 - 20 per cent of all TB cases in HIV - negative patients, while in HIV - positive people, it accounts for 40 - 50 per cent of new TB cases ⁴. The most common sites affected by EPTB are Lymph Node, pleura, GIT, skeletal system, urogenital system and meninges and other organs with a significant case mortality of 25% to 50%⁵. Despite this data research on palpable EPTB is limited possibly because EPTB is less transmissible than pulmonary TB. The wide variety of clinical manifestations, difficulty in obtaining samples from the sites and the paucibacillary nature of the specimens pose challenges in EPTB diagnosis. Further, there are no formal national or international guidelines on EPTB. To address these concerns, Indian EPTB guidelines were developed under the auspices of Central TB Division and Directorate of Health Services, Ministry of Health and Family Welfare, Government of India. The objective was to provide guidance on uniform, evidence - informed practices for suspecting, diagnosing and managing EPTB at all levels of healthcare delivery.⁶

Fine needle aspiration cytology (FNAC) has assumed an important role in evaluation of palpable swellings to rule out EPTB as a possible non invasive procedure to excision biopsy.⁷ FNAC is recognized as a rapid diagnostic technique which is a safe, easy, and cost effective procedure used in resource restricted country like ours for the diagnosis of EPTB. The cytological criteria have been clearly defined as

epithelioid granulomas with or without multinucleated giant cells and caseation necrosis.

AFB staining is a very quick, easy and cheap method for diagnosis of TB but its major drawback is its poor sensitivity especially in EPTB. Culture is considered the gold standard. But conventional solid culture is very time consuming. Newer liquid culture techniques require high technical expertise, and the cost of instrument precludes their use in peripheral laboratories in TB endemic countries⁸. Therefore, the need for new rapid and accurate diagnostic methods has emerged. With the rapid evolution of molecular techniques, a wide variety of nucleic amplification tests (NAATs) such as polymerase chain reaction (PCR), real - time PCR, and loop - mediated isothermal amplification (LAMP), are available for the diagnosis of TB⁹.

The aim of the study is;

- 1) Review of spectrum of presentation of palpable extrapulmonary tuberculosis (EPTB).
- 2) To study the role of various diagnostic techniques like FNAC co - adjuvant with other ancillary microbiological tests in diagnosing EPTB in palpable lumps.
- 3) To evaluate the gaps in various techniques in speedy diagnosis of EPTB.

2. Materials and Methods

Study design: This is a retrospective record based study of diagnosed patients of EPTB of all age groups.

Study area: The study was conducted in the North District Hospital, Mapusa, Goa along with IRL, Goa.

Study period: Data for this study has been obtained for a period of 5 years from April 2016 - March 2021.

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Study population: All patients attending various OPD's of Hospital who were diagnosed for extra pulmonary tuberculosis presenting as a palpable lump.

Study method: 235 cases of palpable lumps diagnosed as EPTB were included. FNAC was performed using 22 - 23Gauge needle and 10ml syringe after taking consent from the patient. In each case a part of aspirate was used for preparing three smears, two for H & E (Hematoxylin & Eosin), one for AFB (Fluorescent staining). Part of the aspirate was subjected to NAAT testing. If the aspirate was inadequate in first stroke, repeat aspiration was done. The smears revealing features of Tuberculous lymphadenitis were grouped into four categories. A. Epitheloid Granulomas without necrosis, B. Epitheloid Granulomas with necrosis, C. Necrosis only and D. Caseous necrosis with neutrophilic infiltrate.

A correlation was performed between the nature of cytologic smears with the percentage of AFB and NAAT positivity. Data regarding patients who had positive cytology and AFB report and negative NAAT result were subjected to a repeat aspirate for NAAT and culture was also analysed.

For this study FNAC findings of patients diagnosed with Tuberculosis were analysed for cytomorphological patterns, frequency of AFB, Nucleic Acid Amplification Test and Culture results wherever available in the Pathology Department of North District Hospital, Cytology register. Pulmonary Tuberculosis data was collected from Pathology Department.

Inclusion criteria

- 1) All patients suspected of EPTB presenting to various OPDs as palpable lumps.

Exclusion criteria

- 1) Patients of Pulmonary TB presenting with EPTB.
- 2) EPTB not presenting as palpable lumps.

3. Results

A 5 years retrospective study was carried out to determine the prevalence of EPTB among patients who presented with palpable lumps examined by FNAC at Pathology Department, North Goa District Hospital. A total of 7466 FNACs were done during this period, out of this 235 cases were diagnosed as Extra Pulmonary Tuberculosis on FNAC. There was a female predominance of EPTB in our study (51.4%), (Refer table 1). The proportion of EPTB was found more in age group of 16 - 45 years. (Refer Table 1). The most affected site was the lymph nodes constituting 92.2% of which cervical lymphadenopathy was the commonest presentation (67.2%) followed by axillary lymphadenopathy (13.2%). EPTB was also found at various locations other than lymph nodes in varying percentages. (Refer Table 2). Aspirates which were purulent and cheesy showed higher percentage of AFB positivity and NAAT positivity. (Refer table 3 and 4). AFB and NAAT test correlation with cytomorphology increases with evidence of necrosis seen on the cytology smears. (Refer Table 5 and 6). Total number of six cases was subjected for culture, out of which one was a recurrence of EPTB with AFB positivity. Culture revealed a

Mycobacterium other than Tuberculosis (MOTT). Other 5 cases were AFB negative and NAAT negative with epitheloid granulomas. MTB was detected on culture in these cases. A comparison of 235 palpable EPTB cases diagnosed was done with 463 PTB cases to analyse the prevalence of EPTB in a District Hospital set up.

Table 1: Age and sex distribution of total EPTB cases

To study the age and sex distribution of EPTB cases in the present study we divided the patients into five categories based on age group (refer table 1).

Age group (in years)	Total	Male	Female
1 - 15	33 (14%)	18	15
16 - 30	88 (37.4%)	35	53
31 - 45	77 (32.8%)	36	41
46 - 60	26 (11.1%)	17	09
>60	11 (4.7%)	08	03
Total	235	114 (48.5%)	121 (51.4%)

Table 2: Site wise distribution of EPTB cases

S. No.	Site of EPTB (palpable lumps)	No. of cases	Frequency
A Lymphadenopathy: -			
1.	Cervical swelling	158	67.2%
2.	Submandibular	08	3.3%
3.	Preauricular	02	0.8%
4.	Bilateral neck swelling	08	3.4%
5.	Generalised	04	1.7%
6.	Axillary swelling	31	13.2%
7.	Inguinal Swelling	02	0.9%
8.	Epitrochlear swelling	04	1.7%
		217	92.2%
B Others: -			
9.	Chest wall swelling	07	3.0%
10.	Hypochondriac swelling	02	0.9%
11.	Paraspinal swelling	03	1.3%
12.	Umbilical swelling	01	0.4%
13.	Breast lump	03	1.3%
14.	Scalp swelling (Occipital)	02	0.9%
	Total	235	100%

Table 3: Distribution based on gross nature of aspirate on FNAC & correlation with AFB

Type of aspirate	Number of cases	AFB Positive	AFB Negative
Cheesy aspirate	41 (17.4%)	25 (61%)	16 (39%)
Cheesy material mixed with blood	106 (45.1%)	63 (59.4%)	43 (40.6%)
Purulent	73 (31.1%)	35 (47.9%)	38 (52.1%)
Blood mixed aspirate	15 (6.4%)	03 (20%)	12 (80%)
Total	235	126 (53.6%)	109 (46.4%)

Table 4: Distribution based on gross nature of aspirate on FNAC & correlation with NAAT testing

Type of aspirate	Number of cases	NAAT: MTb detected	NAAT: MTb not detected
Cheesy aspirate	41 (17.4%)	38 (92.7%)	03 (7.3%)
Cheesy mixed with blood	106 (45.1%)	69 (64.2%)	38 (35.8%)
Purulent	73 (31.1%)	69 (94.5%)	04 (5.5%)
Blood mixed aspirate	15 (6.4%)	03 (20%)	12 (80%)
Total	235	179 (76.2%)	56 (23.8%)

Table 5: Cytomorphologic pattern on microscopy and its correlation with AFB

Cytomorphologic pattern	Total number of cases	AFB Positive	AFB Negative
Epithelioid Granulomas without necrosis	43 18.2%	10 (23.3) %	33 (76.7) %
Epithelioid Granulomas with necrosis	103 43.8%	62 (60.2%)	41 (39.8%)
Necrosis only	55 23.4%	38 (69.1%)	17 (30.9%)
Caseous necrosis with neutrophilic infiltrate	34 14.4%	16 (47.1%)	18 (52.9%)
Total	235	126 (53.6%)	109 (46.4%)

Table 6: Cytomorphologic pattern on microscopy and its correlation with NAAT Testing

Cytomorphologic pattern	Total number of cases	NAAT Positive	Rifampicin Sensitive	Rifampicin Resistance
Epithelioid Granulomas without necrosis	43	25 (58.1%)	24	01
Epithelioid Granulomas with necrosis	103	80 (77.7%)	80	—
Necrosis only	55	51 (92.7%)	49	02
Caseous necrosis with neutrophilic infiltrate	34	23 (67.6%)	23	—
Total	235	179 (76.2%)	176 (98.3%)	03 (1.7%)

4. Discussion

Pulmonary tuberculosis is the most common presentation of tuberculosis. PTB diagnosis and treatment is a main priority for public health and the focus of National TB Elimination Programme. Improved living standard and availability of TB chemotherapy is bringing down the burden of PTB to some extent in our country. At the same time EPTB has a huge impact on mortality and morbidity rate in India and worldwide¹.

FNAC is a simple, easy, non invasive, reliable tool with high sensitivity and first line investigation carried as outpatient procedure for diagnosing palpable EPTB in India, where TB is the major cause of morbidity and mortality. Histopathology is reserved only for patients with high clinical suspicion and negative FNAC¹⁰. In our study the incidence of EPTB constituted 3.14% of all palpable lumps on FNAC (n=7466). EPTB can affect lymph nodes, musculoskeletal system, meninges, genitourinary system, skin, abdomen etc. In our study Cervical lymphnodes were most common site affected (67.2%) followed by axillary lymphnodes (13.1%), submandibular lymphnode (3.4%) bilateral neck swelling (3.3%), chest wall swelling (2.9%), epitrochlear and generalized lymphadenopathy (1.7%) which is concordant with the study done by Shrivastava et al, Priyanka Chand et al and Komanapalli SK et al,^{10, 11, 12}. Other sites of EPTB seen in our study are preauricular lymphnodes, breast, inguinal swelling, epitrochlear lymphnodes, umbilical swelling, hypochondriac swelling, paraspinal swelling and scalp swelling. So any palpable lump should be evaluated for EPTB specially where pus or pus like material is aspirated.

In our study we have seen 66.38% of Pulmonary Tuberculosis and 33.62% of EPTB cases with a ratio of percentage of EPTB to PTB was 1: 2. As per national survey the incidence of EPTB in India is 15% - 20%². As per study done by Shrivastava et al 26.47% of EPTB was reported. The ratio of percentage of EPTB to PTB was 1: 3.6.¹⁰ Our study includes only palpable EPTB and does not include other EPTB like pleura, bone, meninges etc which may be contributing to EPTB and will further increase the incidence of EPTB. The reason for this high percentage positivity may be due to availability of diagnostic facilities in District

Hospital. There is a need to thoroughly investigate the etiology for the same.

The mean age was 34.8 years and male: female ratio was 1: 1.06. As per study by Shilpa G et al there was a male preponderance.¹³ However our study showed a female preponderance, which was concordant with the study done by Priyanka Chand et al and Komanapalli SK et al.

Females tended to reactivate more with EPTB: approximately half of the overall EPTB cases (51.4%), suggesting that female gender may be a protective factor against PTB or a facilitating factor for EPTB. The results of recent studies also support our findings. In a study carried out in Thailand, TB lymphadenitis was found to be higher in females in the adult population.¹⁴ In a lymphadenitis TB study performed in 1940, female predominance in EPTB was associated with hormonal changes.¹⁵

All 235 cases were subjected to AFB staining and NAAT testing. In our study we have seen 53.6% AFB positive cases. Majority of the AFB positive cases have been reported where caseous/purulent material was aspirated on FNA. Our results are concordant with study done by Priyanka Chand et al and Paliwal Nidhi et al^{11, 16}. The major limitation of AFB staining is its poor sensitivity and poor predictive value. It requires a high bacterial count to get a positive result (10^4 - ZN/ 10^3 - FM). In addition hemorrhagic tap further dilutes the already paucibacillary extrapulmonary aspirate and give false negative results.^{1, 16}

NAAT tests have been approved by WHO for diagnosis of PTB and EPTB as they have a quick turnaround time (24 to 72 hours). It can detect low bacterial count and allows rapid initiation of drug susceptibility testing. The sensitivity is variable based on sample type. Negative test may not exclude the diagnosis of TB. In this study NAAT testing was positive in 75.7% cases. Majority of these cases with purulent aspirate were NAAT positive. NAAT test has high specificity in our study which is concordant with Komanapalli et al and M Pai et al^{12, 17}. NAAT sensitivity decreases with aspirates which contain blood as blood acts as a PCR inhibitor. NAAT not only detects MTB but it also helps in rapidly determining patients with Multidrug resistant (MDR) TB which is of prime importance to end the spread of MDRTB and decrease mortality.^{3, 4} 98.3% cases

were Rifampicin sensitive and only 1.7% cases were Rifampicin resistant.

The most common cytomorphologic pattern on microscopy was Epithelioid granulomas with necrosis (43.8%) followed by only necrotic material (23.4%), Epithelioid Granulomas without necrosis (18.2%) and Caseous necrosis with neutrophilic infiltrate (14.4%). A similar pattern was seen in the conducted by Hemlatha A et al and Afeefah Jamsheed et al^{18,19}. 18.2% of non caseating granulomas was seen which needs to be evaluated for the varied etiology. The differential diagnosis of Granulomatous lymphadenitis is wide which includes infectious diseases (bacteria, Virus, fungal, parasitic), malignant disorders mainly lymphoid malignancies, autoimmune diseases like SLE and idiopathic causes like Kikuchi's disease and Sarcoidosis. In our study we got 53.6% AFB positivity and 76.2% NAAT positivity.

TB culture is still important in the diagnosis of TB from paucibacillary samples and is considered as gold standard in diagnosis. It also helps in differentiating non tuberculous mycobacterial infections. However culture cannot be used as a primary diagnostic test in all setups. It requires BSL3 facility and a long time to generate results i. e. upto 6 weeks.¹⁷ In our study TB culture helped in diagnosis of cases which were not detected by AFB and NAAT testing.

Our study had several limitations as it is a retrospective District Hospital based study and hence the findings cannot be generalized to the community. Cumulative risk factors like malnutrition, diabetes, HIV, smoking, chronic liver disease, chronic kidney disease, immunosuppression etc was not known.

5. Conclusion

EPTB is a complex disease and needs a high degree of clinical suspicion due to its ambiguous presenting symptoms. FNAC can be used as a reliable, economical investigating modality for the diagnosis of EPTB presenting as palpable lumps. None of the existing diagnostic techniques are mutually elusive. Co - adjuvant microbiological techniques can be used in rapid diagnosis of EPTB. This will help in directed treatment, decrease morbidity and increase life expectancy. The index TB guidelines by the NTEP represented the start of a process of developing evidence - informed EPTB guidelines in India and this would be further developed over time aiding in timely and correct diagnosis. The rate of EPTB is higher in our study compared to national standards. This needs to be investigated and a prospective large scale epidemiological study can be undertaken to assess the reason for eradication of Tuberculosis in long term.

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