International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN
Original Research Article

A study on Cytomorphological spectrum of Extra pulmonary Tuberculosis in Rural Tertiary Care Hospital

Sonia Hasija[1], Shilpa Garg[*1], Pawan Singh[2], Shivani Kalhan[3], Neerav Saini[4] and Anam Khan[4]

[1]*Assistant Professor, Department of Pathology, SHKM, GMC Nalhar, Nuh, India*
[2]*Associate Professor, Department of Pathology, SHKM, GMC Nalhar, India*
[3]*Professor & Head, Department of Pathology, SHKM, GMC Nalhar, Nuh, India*
[4]*Demonstrator, Department of Pathology, SHKM, GMC Nalhar, Nuh, India*

QR Code

***Correspondence Info:**
Dr. Shilpa Garg
Assistant Professor,
Department of Pathology,
SHKM GMC Nalhar Nuh 221107, India

***Article History:**
Received: 06/02/2018
Revised: 15/02/2018
Accepted: 15/02/2018
DOI: https://doi.org/10.7439/ijbar.v9i2.4617

Abstract

This is estimated that about 1/3rd of world population is infected with mycobacterium tuberculosis & most of the cases in Asia (55%) and Africa (31%) rest in others. Extra Pulmonary Tuberculosis (EPTB) reversed the epidemiological trend over the last several years. EPTB was has been increasing in absolute numbers and in proportions of all reported TB cases. Since very less data is available on EPTB and its distribution especially from this part of India therefore this study has been planned to assess the pattern of EPTB & the role of Fine Needle Aspiration Cytology (FNAC) in diagnosing these cases. This is a retrospective analysis of patients with peripheral lymphadenopathy & swelling on various sites. All the patients had fine needle aspiration. Smears were made, fixed in 95% alcohol and stained with giemsa, hematoxylin and eosin and Zeihl Neelsen (ZN) stains. The study includes 271 cases of extrapulmonary tuberculosis in which patient age range from one year to more than 51 years. Maximum no. of cases falls in the age group between 11 to 20 years. The mean age was 22.6 years. Females (63%) had higher proportion of EPTB than males (37%). Aspirates were mainly from cervical lymph nodes. Other sites were chest wall, breast, thigh, back etc. Macroscopically, 106 (39%) of the aspirates were purulent and 165 (61%) had necrotic material. Microscopy we divide it in three patterns, Pattern A (Epithelioid granuloma without necrosis) (39.11%); Pattern B (Epithelioid granuloma with necrosis) (45.75%); Pattern C (necrosis/neutrophilic aspirate) (15.12%). Staining for acid-fast bacilli was positive in 47 (17.34%) cases. Early diagnosis of extrapulmonary tuberculosis in a resource-limited setting can be achieved with fine needle aspiration cytology technique (FNAC). This will ensure prompt treatment and thus reduce attendant morbidity and mortality.

Keywords: Extra Pulmonary Tuberculosis, Necrosis, AFB.

1. Introduction

Tuberculosis remains an endemic in India. It is the seventh leading cause of death globally. Burden of TB in India is one-fifth (21%) of global incidence. In India, two deaths occur every three minutes from TB.[1] Extra pulmonary tuberculosis (EPTB) is the presence of disease in an organ without obvious involvement of the lungs. The present article focuses on the incidence of extra pulmonary tuberculosis as an emerging and clinically significant disease to be reckoned with in the present era. It also highlights Fine Needle Aspiration Cytology (FNAC) as an inexpensive, less invasive procedure for early diagnosis of such tuberculosis and timely initiation of specific therapy. The percentage of patients with EPTB in tertiary care centres in India varies between 30-50%.[2]

2. Material and Methods

The present study was carried out retrospectively from August 2015 to July 2016 in Department of Pathology, SHKM, Government medical college, Nalhar, Nuh. This study includes 271 cases who has cytomorphological pattern on FNAC was suspicious for tuberculosis. All cytology on extrapulmonary tuberculosis was studied and correlated with the clinical history and presentation. FNAC was performed by the conventional

method using 23 gauze needles. Aspirate was pus like to hemorrhagic in gross appearance. Slide were fixed in 90% alcohol and stained with giemsa & hematoxylin and eosin stain.

Suspicious cytomorphological pattern of tuberculosis:

Pattern A – Epithelioid granuloma without necrosis

Pattern B –Epithelioid granuloma with necrosis

Pattern C – Necrosis/ neutrophilic infiltrate.

Cases presented with EPTB along with pulmonary involvement was considered as pulmonary tuberculosis as per the guideline of WHO and therefore excluded from this study

The most confirmatory diagnostic tool used in this study for the confirmation of extrapulmonary tuberculosis was the demonstration of acid fast Bacilli using Ziehl–Neelsen staining in 20% H_2SO_4. Ziehl–Neelsen staining positivity was analyse in relation to all the patterns. Chi-square test was done to correlate cytomorphological pattern and bacillary positivity. $P < 0.05$ was considered as significant.

3. Results

The study includes 271 cases of extrapulmonary tuberculosis in which patient age range from one year to more than 51 years. Maximum no. of cases falls in the age group between 11 to 20 years.

Table 1: Distribution of cases according to age

Age (years)	Male	%	Female	%	Total	%
0-10	20	7.38	23	8.48	43	15.86
11-20	34	12.54	62	22.87	96	35.42
21-30	27	9.96	45	16.60	72	26.56
31-40	5	1.84	22	8.11	27	9.96
41-50	8	2.95	12	4.42	20	7.38
>51	6	2.21	7	2.58	13	4.79
Total	100	36.90	171	63.09	271	

Out of 271 cases, 250 cases (92.25%) presented with lymphadenopathy and 21 cases (7.74%) presented with swelling to different sites other than lymph node. Out of 250 lymphadenopathy cases, cervical lymphadenopathy was most common lymphadenopathy comprising 206 cases i.e (76.01%) as shown in table 1. Out of 21 cases show which show different site of involvement i.e. 6 cases (2.21%) show swelling chest wall, 3 cases (1.10%) presented with breast lump, (1.10%) lumber region back swelling and other various sites as shown in table 3.

Table 2: Distribution of EPTB cases according to different lymph node involvement

Site of lesion	Number of cases	%
Cervical lymph node	206	76.01
Axillary lymph node	16	5.90
Submandibular lymph node	13	4.79
Inguinal lymph node	7	2.58
Submental lymph node	5	1.84
Supraclavicular lymph node	3	1.10
Total	250	92.25

Table 3: Distribution of different sites of EPTB cases other than lymph nodes

Site of Lesion	Number	%
Swelling chest wall	6	2.21
Breast lump	3	1.10
Thigh	3	1.10
Lumbar area on back	3	1.10
Scrotal	1	.36
Testis	1	.36
Cheek	1	.36
Foot	1	.36
Thyroid	1	.36
Arm	1	.36
Total	21	7.74

Cytomorphological pattern of 271 cases was divided into three pattern as shown in table 3, Pattern A: epitheloid cell granuloma without necrosis it comprises 106 cases (39.11%) out of which only 8 cases show AFB positivity and the relation between pattern A and AFB positivity was statistically significantly by using chi square test. Similarly124 cases (45.75 %) showed Pattern B i.e. epitheloid cell granuloma with necrosis, in which 20 cases (16.12 %) have shown AFB positivity. But in this study this relationship was not statistically significant (P> 0.005). Pattern 3 showed by 41 cases (15.12%) in which 19 cases (45.23%) showed AFB positivity and this relationship was extremely statistically significant as shown in the table 4.

Table 4: Distribution of cases according to the three cytomorphological pattern and its relation with AFB positivity

Group	Number	%	AFB positive	%	P value
Epithelioid granuloma without necrosis	106	39.11	8	7.54	~ 0.0012
Epithelioid granuloma with necrosis	124	45.75	20	16.12	~ 0.7461
Necrosis/ neutrophilic infiltrate	41	15.12	19	45.23	~ 0.0001
Total	271		47	17.34	

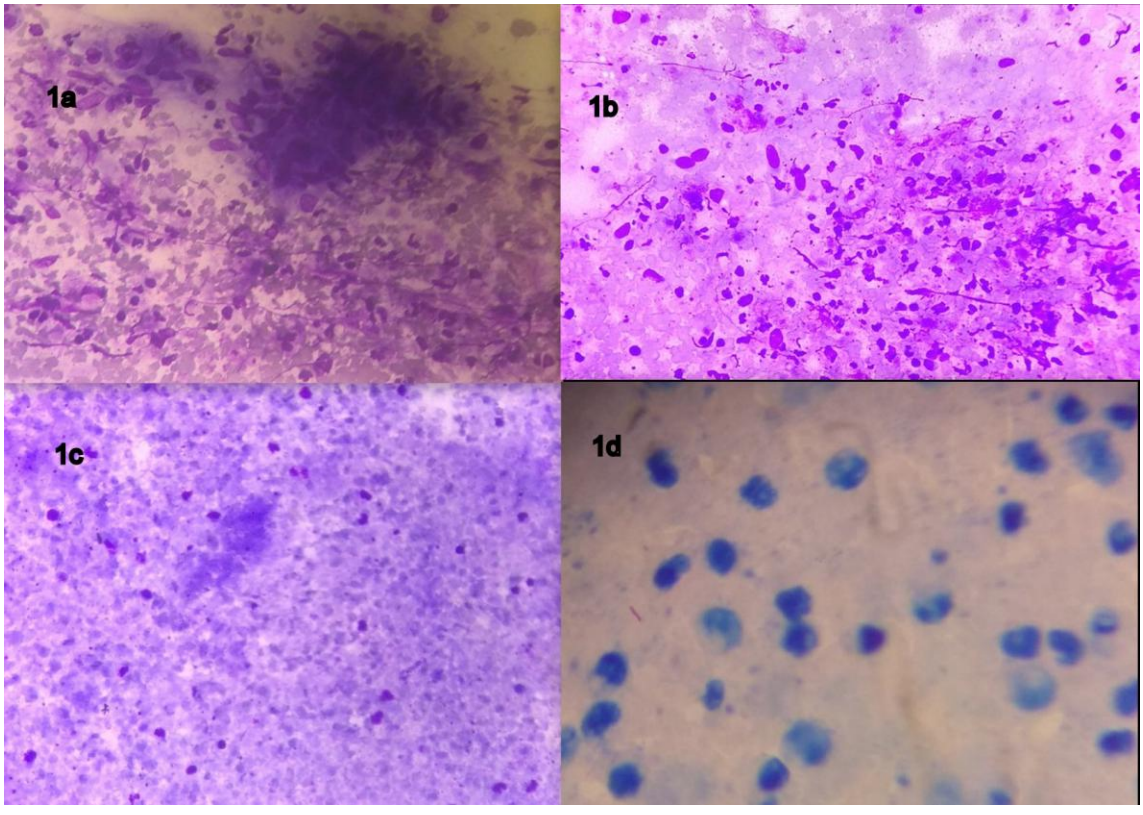

Figure 1a: Pattern A – Epithelioid granuloma without necrosis
Figure 1b: Pattern B –Epithelioid granuloma with necrosis
Figure 1c: Pattern C – Necrosis/ neutrophilic infiltrate.
Figure 1d: AFB positivity Ziehl Neelson (ZN) staining

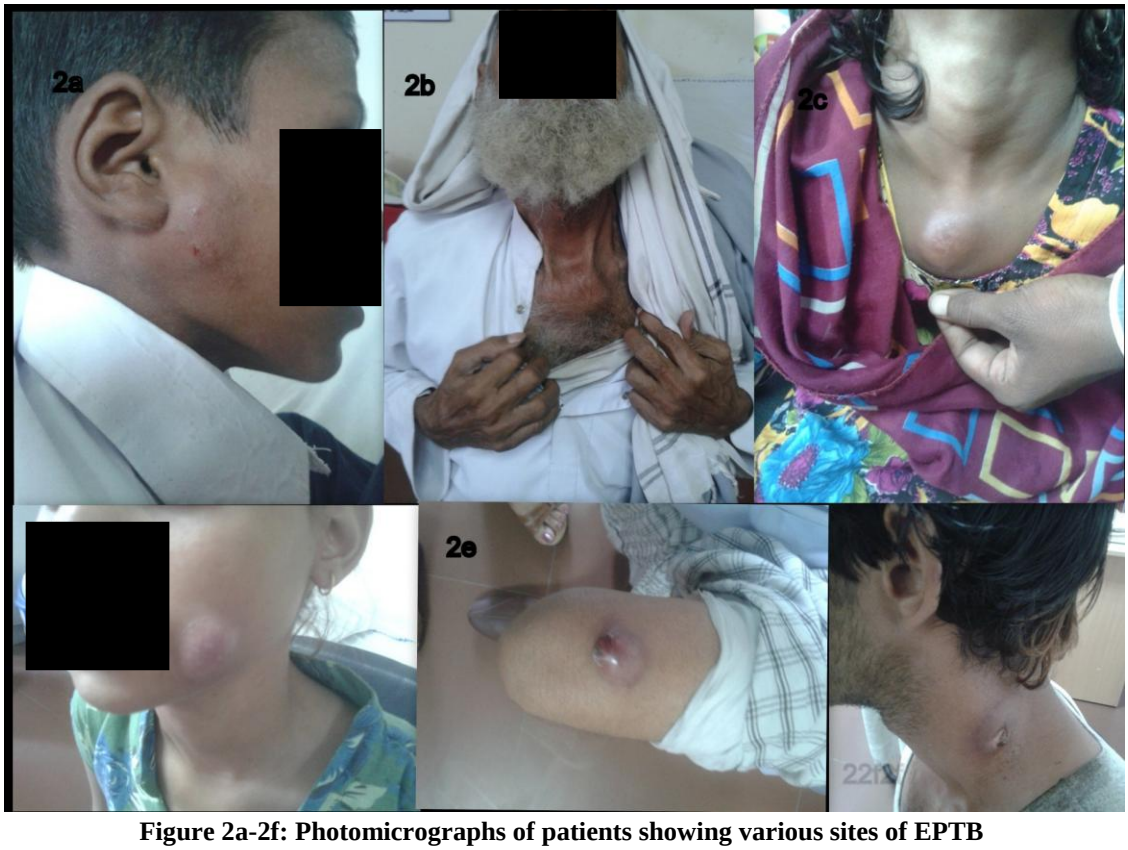

Figure 2a-2f: Photomicrographs of patients showing various sites of EPTB

4. Discussion

Extrapulmonary tuberculosis (EPTB) describes the various conditions caused by Mycobacterium tuberculosis infection of organs or tissues outside the lungs. There are many forms of EPTB, affecting every organ system in the body. Some forms, such as TB meningitis and TB pericarditis, are life-threatening, while others such as pleural TB and spinal TB can cause significant ill-health and lasting disability. 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 1. The estimated incidence of TB in India was 2.1 million cases in 2013, 16 per cent of which were new EPTB cases, equating to 336,000 people with EPTB.[3] Every year 1.98 million cases are reported as tuberculosis from India as compared to global annual incidence of 9.4 million. Extra Pulmonary TB constitutes 15-20% of all cases of TB.[4]

Lymphadenitis is the most common extra pulmonary manifestation of TB seen in nearly 35% of extra pulmonary TB (92.75% in our study). Numerous diagnostic methods are available for detecting extra pulmonary TB such as fine needle aspiration cytology (FNAC), culture, polymerase chain reaction and histopathological examination. FNAC is a routinely done cytological technique in diagnosing tubercular lymphadenitis as it has sensitivity and specificity of 88-96%.[4] FNAC is economical and rapid as compared with culture studies. Culture studies considered as gold standards but it is time consuming and requires skill.

Smears should be stained with the acid-fast fluorochrome dye, auramine-O, which is more sensitive than the conventional Ziehl-Nielsen stain.[5] Rapid probes can be applied to smear-positive sputum specimens to confirm the diagnosis of *M. tuberculosis* (in areas where available).[6] Specimens should then be inoculated into a commercial automated radiometric detection system (BACTEC, Becton Dickson), which is faster and more sensitive than standard techniques using solid medium for the isolation of *M. tuberculosis*.[7] *M. tuberculosis* can be differentiated from commonly isolated nontuberculous mycobacteria by hybridization using nucleic acid probes on the liquid medium.

Mycobacterial blood cultures (preferably using lysis centrifugation techniques) should be performed in all patients in who hematogenous dissemination is suspected.[8] Positive blood cultures in disseminated TB are relatively rare though may be observed in immunocompromised patients, including those with HIV infection.[9]

Most reliable criteria for diagnosing tubercular lymphadenitis is a demonstration of acid fast bacilli (AFB) as demonstrated by Ziehl Neelson (ZN) stain, auramine rhodamine stain and polymerase chain reaction or culture of bacilli from aspirates. In spite of availability of above methods, in our population, with limited resources and high tubercular disease burden presence of epithelioid cell granuloma is considered as an evidence of tubercular lymphadenitis.

In our study patient's age ranged from 2 years to 62 years. Maximum numbers of cases were in the age group of 11-20 years followed by age group of 21-30 years and 0-10 years. This is the most productive age group and thus in directly affects the economic development of country. Adriano *et al*[10] also reported that tuberculosis is a disease that affects mainly young adults. We found the youngest patient was 2yrs and the oldest was 62yrs of age. These figures are in close comparison with Rajwanshi A[11] also reported that age of patient ranged from 1.5 to 72yrs. We observe that there is female preponderance among tubercular lesions with M:F 1:2.27 where as Rajwanshi *et al* founded 1:1.3. A declining trend was seen in the geriatric population. Ahmad *et al* also described similar trend.[12]

In India and other developing countries lymph node tuberculosis continues to be the most common form of extra pulmonary tuberculosis. In our study, at a rural tertiary care center, cervial tubercular lymphadenitis constituted 76.01% of all EPTB aspirates forming the most common cause of lymph node enlargement which is very close comparable with the findings of Shamshad *et al*[12] in which it was 73.6% and Egea[13] reported it as 67.51%. and Shrivastwa *et al*[14] reported it was 69.41%. Other sites involved were chest swelling 2.21%, breast, thigh lesion and back swelling falls in the same percentage of Shrivastwa *et al*[14] reported. In bones TB was found in 1.83% cases followed by scrotum, testis, preauricular area, foot, thyroid, arm.

Limitations of our study include deficiencies in the secondary databases including, possible missing or incomplete data, and potential biases and errors during data entry. We analyzed data for patients with extrapulmonary forms of TB. Additional studies of patients with combined pulmonary and extrapulmonary TB are needed to understand the characteristics of such patients.

The main limitation of the study is that the being a retrospective, hospital-based study, the findings cannot be generalized to the community, but it gives valuable information regarding trend of EPTB cases in Mewat and the associated risk factors. Although associated HIV infection is known risk factors for increasing EPTB infection, HIV status was not known in substantial number of EPTB cases. The small sample size of this study limits the calculation of the true prevalence of EPTB for the state of Haryana. Furthermore the study being record based, it did not include information of other possible risk factors such as nutritional status, smoking, alcohol intake, drug addiction, vitamin D3 status etc. In addition, it is believed that EPTB might be underreported due to its difficult

diagnosis, which requires a high degree of clinical suspicion and biopsy or culture for confirmation. Strengths of our study include its national scope, the use of a hierarchical logistic regression model, and its new contribution to the knowledge about EPTB in rural area like Mewat.

As already mentioned early diagnosis of extrapulmonary tuberculosis can also be achieved with polymerase chain reaction (PCR) for the detection of mycobacterium DNA. This facility is however not readily available in most developing countries where the disease is prevalent, and where available, the cost is prohibitive to the patients. It is advocated that in countries where tuberculosis is endemic, like in India, treatment can be instituted without the need for excisional biopsy if FNA results show eosinophilic granular material (caseation).[15]

Early diagnosis of extrapulmonary tuberculosis in a resource limited setting can be achieved with FNAC technique. This will ensure prompt treatment and thus reduce attendant morbidity and mortality.

5. Conclusion

Fine needle aspiration biopsy (FNAB), a simple and safe out-patient procedure. Knowledge in the varied cytomorphological spectrum of suspicious TB smears is necessary for diagnosis and follows up of patients. The purulent aspirate showed significant AFB load mandates ZN staining. Early diagnosis of EPTB in resource limited setting can be achieved with FNAC technique. This will ensure prompt treatment and reduce morbidity and mortality.

Conflict of interest: None

References

[1]. Dandapat Z.M. C., Mishra B. M., Dash S. P., and Kar P. K., "Peripheral lymph node tuberculosis: a review of 80 cases," *British Journal of Surgery*, 1990; 77(8): 911–912.

[2]. Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res* 2004; 120:316-53.

[3]. Lau S. K., Kwan S., Lee J., and Wei W. I., "Source of tubercle bacilli in cervical lymph nodes: a prospective study," *Journal of Laryngology and Otology*, 1991; 105(7): 558–561.

[4]. Hemalatha A, Shruti PS, Kumar UM, and Bhaskaran A. Cytomorphological Patterns of Tubercular Lymphadenitis Revisited. *Ann Med Health Sci Res*. 2014; 4: 393–396.

[5]. Hooja S, Pal N, Malhotra B, Goyal S, Kumar V, Vyas L. Comparison of Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. *Indian J Tuberc*. 2011; 58(2): 72-6.

[6]. Yon Ju Ryu. Diagnosis of Pulmonary Tuberculosis: Recent Advances and Diagnostic Algorithms. *Tuberc Respir Dis* (Seoul). 2015; 78(2): 64–71.

[7]. Siddiqi S, Ahmed A, Asif S, Behera D, Javaid M, Jani J *et al*. Mycobacteriology and Aerobic Actinomycetes Direct Drug Susceptibility Testing of Mycobacterium tuberculosis for Rapid Detection of Multidrug Resistance Using the Bactec MGIT 960 System: a Multicenter Study. *J Clin Microbiol* 2012; 50: 435-440.

[8]. Parikh HR, De AS, Baveja SM. Comparison of the lysis centrifugation method with the conventional blood culture method in cases of sepsis in a tertiary care hospital. *J Lab Physicians*. 2012; 4:89-93.

[9]. Sharma SK, Mohan A, Sharma A. Challenges in the diagnosis & treatment of miliary tuberculosis. *Indian J Med Res*. 2012 May; 135(5): 703-30.

[10]. Adrian O, Jennifer C, Philip CH, Leah C, Gonzalez MW, Robert MJ, Charles LD. Molecular Epidemiological Assessment of Extra pulmonary Tuberculosis in San Francisco. *Clin Infe Dis*. 2004; 38(1): 25–31.

[11]. Rajwanshi A, Bhambani S, Das DK fine needle aspiration cytology diagnosis of tuberculosis *Diagn cytopathol* 1987; 3; 13-16.

[12]. Ahmad SS, Akhtar S, Akhtar K, Naseem S, Mansoor T, Khalil S. Incidence of tuberculosis from study of fine needle aspiration cytology in lymphadenopathy and acid fast staining. *Indian J Community Med.* 2005; 30:63–5.

[13]. Egea AS, Gonzalez MAM *et al*. Usefulness of light microscopy in lymph node fine needle aspiration biopsy. *Acta Cytologica* 2002; 46; 368-69.

[14]. Shrivastava A, Koshti A, Neelkamal Kapoor, Nigam RK. Fine Needle Aspiration Cytology -an Important Tool For Diagnosis Of Extra Pulmonary Tuberculosis. *Int J Biol Med Res.* 2013; 4(1): 2762-2764.

[15]. Koo V, Lioe TF, Spence R. Fine needle aspiration cytology (FNAC) in the diagnosis of granulomatous lymphadenitis. *Ulster Med J.* 2006; 75:59–64.