

**HISTOPATHOLOGY AS A PRIMARY INVESTIGATION TOOL IN THE DIAGNOSIS OF EXTRA-PULMONARY TUBERCULOSIS****Dr. P. Venkata Ramana Babu***

Professor And HOD, Department Of Pathology, Kurnool Medical College, Kurnool. Andhra Pradesh. *Corresponding Author

Dr. Lakshmi. V

Post Graduate Student, Department of Pathology, Kurnool Medical College, Kurnool. Andhra Pradesh.

ABSTRACT **Introduction:** Tuberculosis(TB) is an ancient disease.It is second only to HIV as a cause for fatal infection in the developing world and remains a major global public health challenge. The term 'extra-pulmonary tuberculosis' (EPTB) refers to tuberculosis infection occurring in all systems other than lungs. The diagnosis of extra-pulmonary TB is challenging, often requiring biopsy. Histopathological examination is a simple, safe, inexpensive method with wide acceptance and used as a first-line diagnostic procedure for diagnosis of extra-pulmonary tuberculosis. **Aim:** The main aim of the study was to diagnose the clinically suspected surgical specimens of extra-pulmonary tuberculosis by histopathological examination. **Setting And Design:** This was a retrospective study done in the Department of Pathology, Kurnool Medical College, Kurnool. **Materials And Methods:** Study period was for 2 years (January 2022 to December 2023). For all clinically suspected surgical specimens of extra-pulmonary tuberculosis, grossing was done, processed; Haematoxylin and Eosin stain was done along with Ziehl-Neelsen stain, wherever necessary. Reporting was done by light microscopy. Data was collected and entered in MS excel sheet. **Results:** Among 150 clinically suspected specimens of extra-pulmonary tuberculosis studied, 114(76%) were reported as extra-pulmonary tuberculosis. Of these, 26(22.81%) were caseating tuberculous lymphadenitis, comprising the predominant manifestation of extra-pulmonary tuberculosis, followed by genitourinary tuberculosis accounting for 25 cases (21.93%). Tuberculosis of abdomen, head and neck, central nervous system, pleura, musculoskeletal system, skin and breast were 22(19.30%), 12(10.53%), 8(7.02%), 7(6.14%), 6(5.26%), 6(5.26%) and 2(1.75%) respectively. **Conclusion:** Histopathological examination is the common and most important primary diagnostic test in clinically suspected cases of extra-pulmonary tuberculosis and there was a high degree of positive outcome with a final histopathological report.

KEYWORDS : Extra-pulmonary tuberculosis, Histopathology, Diagnosis, Biopsy.**INTRODUCTION**

Tuberculosis is a communicable disease that is a major cause of ill health, causing 2 million deaths a year worldwide^[1] and the leading cause of death from a single infectious agent. TB is caused by the bacillus *Mycobacterium tuberculosis* (MTB), which is spread when people who are sick with TB expel bacteria into the air; for example, by coughing.

The prevalence of TB has raised in both immunocompetent and immunocompromised individuals and it can affect any organ. Tuberculosis infection of any part of body other than lung parenchyma is defined as extra pulmonary tuberculosis (EPTB). It can present in various parts of the body, including musculoskeletal system, central nervous system, lymph nodes, pleura, skin, abdomen, eyes, head and neck region, genitourinary system, heart, breast and more.

While pulmonary TB (PTB) is the most recognised form, currently, extra-pulmonary presentations form a major proportion of new cases. Globally, EPTB accounts for approximately 15%–20% of all TB cases, though this proportion varies by region. India, with its high incidence of TB, shoulders a considerable burden of EPTB. The Nikshay platform reported over 2.4 million TB cases in India in 2022, with EPTB constituting 24% of these cases^[2].

Diagnosis of EPTB can be baffling, compelling a high index of suspicion owing to pauci-bacillary load in the biological specimens. Delay in diagnosis and treatment initiation lead to poor prognosis and sequelae in majority of the cases. The diagnosis of EPTB often requires biopsy. Histopathological examination is one of the classical diagnostic methods which is accepted widely for the diagnosis of EPTB.

Aim

1. To analyse the histopathological features of clinically suspected specimens of extra-pulmonary tuberculosis.
2. To estimate the percentage of cases that are positive and negative for extra-pulmonary tuberculosis on histopathological examination.
3. To know the most common age distribution, gender, site of extra-pulmonary tuberculosis.

MATERIALS AND METHODS

It is a retrospective observational study done in the Department of Pathology, Kurnool Medical College, Kurnool. Based on the data collected from the registers from the period of January 2022 to December 2023.

Inclusion Criteria:

All the specimens suspected as extra-pulmonary tuberculosis clinically with the submitted data of clinical history, age, gender, site to the Department of Pathology, Kurnool Medical College, Kurnool.

Exclusion Criteria :

1. Inadequate biopsy samples.
2. Suboptimal preserved samples.
3. Cases that don't contain adequate data for the study.

Sample Size : 150 specimens.

Statistical Analysis :**Table No.1 - Site Specific Distribution Of Cases**

S.no.	Site	No.of cases	Percentage
1	Lymph nodes	26	22.81
2	Genitourinary system	25	21.93
3	Abdomen	22	19.30
4	Head and neck	12	10.53
5	Central nervous system	8	7.02
6	Pleura	7	6.14
7	Musculoskeletal system	6	5.26
8	Skin	6	5.26
9	Breast	2	1.75
	TOTAL	114	

Table No.2 - Age And Sex Distribution Of Cases

S.no	Age group	Male	Female	Total
1	1-10	7	0	7
2	11-20	11	9	20
3	21-30	19	23	42
4	31-40	8	9	17
5	41-50	4	7	11
6	51-60	4	7	11
7	Above 60	2	4	6
	Grand Total	55	59	114

OBSERVATION AND RESULTS

A total of 114 cases of extra-pulmonary tuberculosis were included in the present study. A histopathological diagnosis based on gross, microscopic examination was done and correlated with other relevant investigations .Table No.1 illustrates the distribution of EPTB cases in terms of various sites involved.It can be concluded from Table No.1 that the highest number of cases of EPTB, i.e., 26 were reported in the

lymph nodes (22.81%) followed by 25 cases (21.93%) in the genitourinary system. 10 cases (9.71%) were reported in the male genital system in which 5 cases involved the testis and others involved the scrotum (Fig. 1a). Of 10 cases reported in female genital tract, 7 cases were reported in the endometrium. A single case of ovary involvement was seen. The case of EPTB in the ovary was clinically interesting as it had a coexistent pathology of dermoid cyst, the wall of which showed tubercles. The biopsy was processed and it showed well formed epithelioid granulomas along with caseating necrosis and Langhans giant cells (Fig. 1b). Other rare sites to get involved were fallopian tube and vulva. 5 cases (4.39%) of tuberculosis were reported in kidney. Other sites where EPTB was diagnosed were intestine, omental tissue (22 cases/19.30%), where histopathological examination revealed well formed caseating epithelioid cell granulomas. 12 cases (10.53%) were observed in the head and neck region with one case each reported in eyelid, middle ear, oral vestibule and thyroid. Histopathology of the excised tissue showed multiple epithelioid cell granulomas. Acid Fast Bacilli were demonstrated. 8 cases (7.02%) of EPTB involving the central nervous system were observed, which revealed well defined epithelioid cell granulomas with caseating necrosis. Ziehl-Neelsen staining for acid fast bacilli came positive. 7 cases (6.14%) showed pleural involvement. 6 cases of tuberculosis involving the musculoskeletal system (5.26%) at sites such as bones, joints, tendon were reported. The various subtypes of cutaneous tuberculosis that were observed in our study were lupus vulgaris (3 cases) (Fig. 1c), tuberculosis verrucosa cutis (2 cases) and scrofuloderma (1 case). 2 cases of EPTB were seen in breast biopsy specimens (1.75%) in which both cases showed positivity for Acid Fast Bacilli (Fig. 1d). The data was also analysed regarding the age and sex distribution and it was observed that in both the sexes, maximum number of cases i.e. 42 (36.84%) belonged to the age group of 21 to 30 years, followed by 11-20 years age group with 20 cases falling in that group. The minimum number of cases (6) belonged to more than 60 years in both the sexes. In terms of sex distribution, there were almost equal cases in both the sexes with a slight preponderance of females. The ratio of M:F ratio being 1:1.07. The data has been summarised in Table No.2. Diagnosis of tubercular pathology of the various sites on histopathology was followed by correlation with other investigations of the patient. Pulmonary involvement was ruled out and follow up was done to term the patient as a case of EPTB. The other diagnostic techniques besides histopathology that were also carried out during the work up for extra-pulmonary tuberculosis. Modified ZN staining was employed in all the biopsies. It came out positive in 80 cases. While radiological methods like X ray, ultrasound, CT scan were done in 96 cases only. Other diagnostic techniques used were biochemistry, culture on LJ media and PCR.

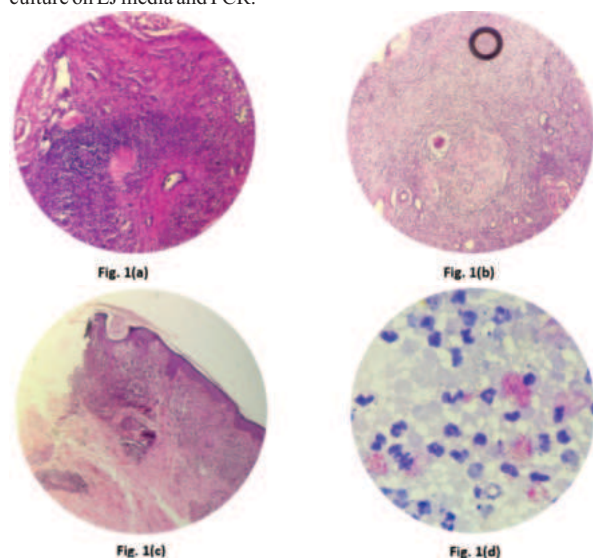


Fig. 1: Microscopic pictures: (a) section from scrotal tissue showing well formed epithelioid granuloma with caseous necrosis (H&E 400X); (b) section from ovary showing granuloma formation with Langhans giant cells (H&E 400X); (c) section from skin showing epithelioid cell granuloma in the dermis (H&E 100X); (d) Ziehl Neelsen staining showing positivity for Acid Fast Bacilli (400X)

DISCUSSION

The causative organism of Tuberculosis is *Mycobacterium*

tuberculosis. Inhalation is the most common route of infection and the predominant clinical form is pulmonary tuberculosis. Tuberculosis remains an endemic disease and is the seventh leading cause of death globally^[3]. Resurgence in Tuberculosis cases has been recorded all over the world with more than 8 million people estimated of being infected with the disease^[4]. Extra pulmonary tuberculosis [EPTB] is defined as Tuberculosis infection of any part of body other than lung parenchyma. Before the advent of HIV pandemic, majority of tuberculosis cases (85%) were pulmonary, and only 15% were extra pulmonary^[5,6]. In HIV positive cases, EPTB accounts for more than 50% of all cases of tuberculosis^[7]. A variety of sites can be involved by EPTB and it may mimic various disease processes. Types of EPTB that have been reported all over the world are lymphatic, pleural, skeletal, central nervous system, pleural, ocular, and pancreatic. The other cases of extra pulmonary tuberculosis include genitourinary and cerebral tuberculosis^[8].

Extra-pulmonary tuberculosis usually poses a diagnostic challenge. It is less familiar to many clinicians as it is not often encountered in routine clinical practice. Patients with EPTB may manifest with constitutional symptoms which are not organ specific, such as fever (37-80% cases), anorexia, weight loss, weakness, night sweats, malaise and fatigue^[9]. At times, when the disease is located at an obscure or occult site, pyrexia of unknown origin (PUO) can be the chief presenting complaint.

Diagnosis of EPTB is difficult at times. No single test can lead to a definitive diagnosis. A variety of direct and indirect methods have to be used. The direct methods in which the bacilli and its products can be demonstrated include Ziehl Neelsen staining, fluorescent microscopy, Lowenstein Jensen culture, BACTEC culture system, Polymerase chain reaction, Antigen based serology, molecular methods, immunohistochemistry and immunocytochemistry, whereas the indirect methods include histopathology which offers an advantage of differential diagnosis, cytology, antibody based serology, skin tests, interferon release assay, adenosine deaminase assay^[10]. Among the indirect methods, histopathology of tissue along with ZN staining and culture for *Mycobacterium Tuberculosis* is considered the investigation of choice for the diagnosis of EPTB. Precise localisation of the lesion and adequate biopsy has been further made easier with the advent of computerised tomographic scan, magnetic resonance imaging, laparoscopy, endoscopy^[11].

In our study, age and sex distribution data showed that EPTB was almost equal in both the sexes with a slight predilection for females (male to female ratio 1:1.07). It was also observed that the majority of patients affected were in the younger age group (21-30 years) followed by 11-20 years. This correlates with other similar study conducted by Sreeramareddy et al^[12] where the ratio for sex distribution was 1.07:1 and younger age group was more affected with 40.9% of the total cases occurring in <25 years age group. Chandir et al^[13] reported maximum number of EPTB cases in 15-29 years age group in Pakistan. These results are comparable with our study. On the other hand, in the same study, the female to male ratio was 3:1, much greater than the present study. It can be attributed to the fact that Pakistani women suffer from Vitamin D deficiency which has been documented as one of the risk factors for tuberculosis^[14]. Studies have been carried out regarding the role of socio economic as well as cultural factors determining gender differentials in tuberculosis. Various determinants including, nutrition, educational status, compliance with treatment, fear and stigma associated with tuberculosis have been found to play an important role^[15].

Amongst the distribution of sites involved by EPTB, lymph nodes (22.81%) was the most frequent site affected, followed by genitourinary system (21.93) and abdomen (19.30%). In the present case series, it was also observed cases of extra-pulmonary tuberculosis with unusual presentations especially when EPTB presented at rare sites. Such cases were clinically interesting as EPTB was not suspected clinically in any of these cases. We reported cases in sites such as vulva, ovary, oral vestibule, and scrotum.

In all the cases of EPTB, patients responded well to the anti-tubercular therapy. Clinical, serological, radiological and histopathological correlation has to be made for arriving at a definitive diagnosis of EPTB. Histopathology plays a major role in such cases.

While comparing the present study with the previous studies (Table

No. 3), it is observed that, sites of localization of EPTB varies from place to place. In a study conducted by Sreeramareddy et al,^[12] the most common site of EPTB was the lymph nodes (42.6%) followed by the peritoneum and/or intestines (14.8%), then bones and/or joints (12.4%). While in the study conducted by Chandir et al^[13] the frequency of EPTB cases by site was highest in lymph nodes (35.6%), followed by spine (26.3%). Similarly, in a study conducted by Noertjojo et al^[16], the genitourinary system and the skin were the common sites affected, whereas in a study carried out by Kang W et al^[17], pleura was the most common site.

Table No.3 - Comparison Of Present Study With The Previous Studies

S. No.	STUDIES	MOST COMMON AGE GROUP AFFECTED	MALE : FEMALE RATIO	MOST COMMON SITE INVOLVED
1	Sreeramareddy et al ^[12]	< 25 years	1.07 : 1	Lymph node
2	Chandir et al ^[13]	15 - 29 years	1 : 3	Lymph node
3	Noertjojo et al ^[16]	< 30 years	1 : 2	Pleura
4	Kang W et al ^[17]	15-24 years	1 : 1.51	Pleura
5	Present study	21-30 years	1 : 1.07	Lymph node

CONCLUSION

EPTB is increasingly becoming an entity that poses a diagnostic dilemma for the clinicians. The clinicians must be aware of the different sites that can be affected including the rarely involved sites. In a developing country like India, extra-pulmonary tuberculosis should always be considered as a differential diagnosis. Definitive diagnosis of tuberculosis involves demonstration of M.tuberculosis by histopathological, microbiological, cytopathological and molecular methods. Histopathology, in particular, assumes great importance when EPTB involves obscure occult sites. It is one of best methods to diagnose EPTB especially when combined with tissue culture and ZN staining. Molecular diagnostic techniques are also used for rapid and accurate detection of tuberculosis. It came out in our study that females and younger age group population (21-30 years followed by 11-20 years) are at risk for EPTB. Specific programs can be formulated by the health care providers targeted at reducing its incidence in females and youngsters. In a developing country like India with a high burden of pulmonary as well as extra-pulmonary tuberculosis, it is important that more researches be carried out on this topic. This would ultimately result in overall decreased morbidity and mortality of EPTB.

REFERENCES

- Gheorghita, Valeriu & Radu, Adelina & Ciobotaru, Ana & Ion, Stefan & Socoluc, Claudiu & Bălănescu, Laura & Caruntu, Florin. (2017). Extra-pulmonary tuberculosis in immunocompetent patients: Case series and mini review of the literature.
- India TB Report 2023. Central TB Division, Ministry of Health and Family Welfare. Available from: <https://reports.nikshay.in/Reports/TBNotification>. [Last accessed on 2023 Sep 15]
- Maher D, Chaulet P, Spinaci S, Harries A. Treatment of tuberculosis: guidelines for national programmes. Geneva: World Health Organization; 1997.
- Zenebe, Y.; Anagaw, B.; Tesfay, W.; Debebe, T.; Gelaw B. BMC Research Notes. 2013, 6, 6-21. DOI: 10.1186/1756-0500-6-21.5. Farer LS, Lowell LM, Mendor MP. Extrapulmonary tuberculosis in the United States. Am J Epidemiol. 1979;109:205-17.
- Fanning A. Tuberculosis: Extrapulmonary disease. CMAJ. 1999;160:1597-603.
- Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. Am Fam Physician 2005;72:1761-8.
- Kandola P, Meena LS. Extra pulmonary tuberculosis: Overview, manifestations, diagnostic and treatment techniques. Adv. Mater. Rev. 2014;1(1):13-19.
- Arango L, Brewin AW, Murray JF. The spectrum of tuberculosis currently seen in a metropolitan hospital. Am Rev Respir Dis. 1978;108:805-12.
- Santos JB, Figueiredo AR, Ferraz CE, Oliveira MH, Silva PG, Medeiros VLS. Cutaneous tuberculosis: diagnosis, histopathology and treatment-Part II. An Bras Dermatol. 2014;89(4):545-55.11. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res. 2004 Oct;120:316-53.
- Sreeramareddy CT, Panduru KV, Verma SC, Joshi HS, Bates MN. Comparison of pulmonary and extrapulmonary tuberculosis in Nepal- a hospital-based retrospective study. BMC Infect Dis 2008;8:8.
- Chandir S.; Hussain H.; Salahuddin N.; Amir M.; Ali F.; Lotia I, Khan A. J J Pak Med. Assoc. 2010;60:105-9.
- Davies PD. A possible link between vitamin D deficiency and impaired host defence to Mycobacterium tuberculosis. Tubercle 1985;66:301-6.
- Hudelson P. Gender differentials in tuberculosis : the role of socio-economic and cultural factors. Tuber Lung Dis. 1996 Oct;77(5):391-400.
- Noertjojo K, Tam CM, Chan SL, Chan-Yeung MM. Extrapulmonary and pulmonary tuberculosis in Hong Kong. Int J Tuberc Lung Dis. 2002;6:879-86.
- Kang W, Yu J, Du J, Yang S, Chen H, et al. (2020) The epidemiology of extrapulmonary tuberculosis in China: A large-scale multi-center observational study. PLOS ONE 15(8): e0237753. <https://doi.org/10.1371/journal.pone.0237753>.