https://doi.org/ 10.33472/AFJBS.6.5.2024. 308-320

African Journal of Biological Sciences

Histopathological studies and Cellular changes of *Mycobacterium tuberculosis* in Extra Pulmonary Tuberculosis in tertiary care Hospital in India

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Article History
Volume 6, Issue 5, Apr 2024
Received: 10 Apr 2024
Accepted: 17 Apr 2024
doi: ***10.33472/AFJBS.6.5.2024. 308-320***

ABSTRACT

Extra Pulmonary Tuberculosis is a airborne bacterial illness caused by Mycobacteriumtuberculosis. The pathogen remains latent in lungs known as Latent TB infection. After infecting lymph nodes, the bacteria eventually enters the bloodstream, where it is distributed to various organs and results in Extra Pulmonary Tuberculosis (EPTB). In the entire world, EPTB is the most prevalent illness.In Mysore, most so in nonrural Mysore (2020), which has the third-highest number of EPTB cases in Karnataka, there is a excessive incidence burden EPTB. For the purpose of identifying the bacterium, fundamental staining procedures like the Hematoxylin and Eosin staining method and the Acid-fast staining method were used. 250 samples of mycobacterium tuberculosis-positive, formalin-fixed, tissue that had been paraffin-embedded was gathered and stained histopathologically, 230 samples revealed the presence of EPTB; the cervical region had the most lymph node swelling (176 cases), followed by the submandibular (40 cases), supraclavicular (18 cases), multiple lymph node (8), axillary (6), inguinal

node swelling (2) cases. Despite the primary anti-TB course of action being 99% effective, drug resistance is seen to be increasing gradually. The current research on MTB detection using H&E staining techniques will support the important management of tuberculosis, together with socioeconomic status. So, the study explains about the identification of the EPTB, through the H & E staining technique is preferred for the better identification of the MTB.

Keywords: Tuberculosis, Extra Pulmonary Tuberculosis Cases , Extra Pulmonary Tuberculosis, Lymph node sites, Histopathology, Diagnosis.

I. INTRODUCTION

The top 10 diseases that cause death include co-morbidities like HIV/AIDS, and the main important factor for death causing includes tuberculosis (TB), which is an airborne and easily spread disease that exhibits the significant reason for weakness [1, 2]. TB is caused by a weak immune system in the patient, making it one of the top 10 diseases that cause death. It Mycobacterium tuberculosis, which causes the disease, spreads when TB-positive individuals remove airborne microorganisms, such as through hacking [3, 4,11]. It frequently affects the lungs, however it can also affect other bodily organs known as EPTB. M. tuberculosis infection affects one-fourth of the populace, putting them at risk of getting TB disease [9, 10,12, 13]. After an early diagnosis and six months of therapy with first-line anti-infection medications, the majority of persons with TB can be relieved and some may not recover, which lowers the risk of contamination spreading before it occurs [5, 6,7]. It can also be directed towards lowering the prevalence of health-related risk factors for TB, such as HIV/AIDS, diabetes, smoking contamination, and so forth; measures for people to protect themselves from an inactive TB infection; and multi-sectoral movements on more comprehensive factors influencing TB disease and illness. Yearly, there are more occurrences of TB, and as a result, more people die from TB-related causes [8,14, 15].

Extra pulmonary tuberculosis (EPTB) (extra = outside of, pulmonary = affecting the lung) is a acute pulmonary infection that causes pulmonary tuberculosis in the Infectious and hosts transfer through various tissues [25, 26]. Along with lesions, affected organs include the bronchus, lungs, pleura, and intrathoracic bronchopulmonary lymph nodes, meningitis, bones, and joints, as well as the larynx, kidneys, pericardium, and abdominal sites. EPTB occurs in about 15–25% of active cases [27, 28]. People with immune system deficiencies and paediatric types are more likely to develop EPTB. Additionally, patients with compromised immune systems are more vulnerable to EPTB, which accounts for 50% of cases [29]. According to the RNTCP's categorization system, EPTB is categorised as TB of organs other than the lungs, such as the pleura, lymph nodes, kidney, genitourinary tract, skin, bones, belly, joints, CNS, and meninges [30].

Different portions of the EPTB spectrum are observed as the pathogen spreads to various body organs. Focused diagnosis and administration are improved by early diagnosis and appropriate epidemiological investigation [35, 36, 37]. It is estimated that 10-25 % of TB contaminations worldwide result in EPTB. Doctors typically first rule out alternative reasons for the symptoms because EPTB is less common. For instance, a hyperextended lower leg with TB in the joint will always result in pain in the right lower leg.

Additionally, EPTB frequently resides in difficult-to-reach body regions, such as the liver, which is located in the middle of the body and cannot having productive reached or examined [38]. With EPTB, even a small number of TB bacilli frequently results in terrible harm. [39]. This is in contrast to lung TB, where the bacilli can persist and grow for an extended period before actually harming the tissue. The most well-known kinds of EPTB typically affect people with HIV. There are numerous varieties, including Disseminated TB, Lymphadenitis. Tuberculous pleural effusion, adrenal TB, bone and Skeletal TB, CNS TB, urinary TB, and others.

Additionally insensitive is acid-fast bacillus (AFB) smear positive [22]. Although it can also be observed in other infectious and non-infectious disorders, chronic granulomatous inflammation on tissue histology supports a diagnosis of probable tuberculosis in AFB-negative, culture-negative patients [23]. *Mycobacteriumtuberculosis* complex DNA is simultaneously found using the novel automated point-of-care real-time nucleic acid amplification test (NAAT) called GeneXpert. It was conditionally recommended over traditional diagnostics (microscopy, culture, and histopathology) in 2014 despite having very low-quality data for increasing case detection in EPTB. The World Health Organisation (WHO) gave it their seal of approval in 2010 for the quick diagnosis of pulmonary tuberculosis (PTB).Although there is limited information on how well lymph node specimens from places with a high TB prevalence may be used for diagnosis, results for stomach aspirate, urine, and stool are encouraging[22].

This study focused on patients with clinically suspected lymph node TB and sought to determine whether there was any correlation between histopathological and microbiological findings in those with extrapulmonary TB. The GeneXpert assay for Mycobacterium tuberculosis direct detection and virulence gene identification from lymph node tissues was also meant to be evaluated for its diagnostic effectiveness. In India, the control programme has 11–15% of EPTB (2020), of which more greater than 6% is lymph node TB. More than 75% of the total EPTB cases have lymph node infection. Other infections that are associated with extrapulmonary tuberculosis include those in the abdomen, skin, central nervous system, kidney, and many more.

II. METHODS AND MATERIAL

Study setting and Participants: The present study was a conducted form April 2017 to December 2020, in Department of Pathology, JSS Hospital Mysore. Where all the EPTB suspected patients were involved (n=250). Lymph nodes (cervical, inguinal, and axillary) measuring >1 cm in greatest diameter and lasting for more than 1 month were taken from patients in the >8 to 60-year-old age group, with or without the presence of a fever, eating disorders, and loss of weight as constitutional signs. In the study, all cases identified by microscopic analysis of the histopathological sections as having tuberculosis in organs other than the lungs were included. The study only included extrapulmonary tuberculosis (EPTB) samples; lung samples were excluded since, according to DTC criteria, people who tested positive for both pulmonary and extrapulmonary tuberculosis were believed to have the infection.

Study Procedures: For the examination of the biological tissues mainly histopathology techniques are practiced, through the microscopic observation. The histopathology mainly includes biopsy samples, where the small paraffin sample were involved for observation of MTB, this mainly helps pathologists for the diagnosing the disease. Hematoxylin and Eosin (H&E) staining was performed after regular processing on biopsies obtained from the pathology department of the JSS Hospital. In every instance, modified Acid-Fast Bacilli were used to help identify Mycobacterium tuberculosis. For the most accurate identification of the EPTB, further investigations including serology, imaging studies like chest radiography, age, gender, chest X-ray, different locations of the lymph nodes, clinical aspects (symptoms), sputum examination, PCR, etc. were associated with the Histopathological diagnosis.

Laboratory Methods: All the specimens that had been collected and formalin-fixed underwent hematoxylin and eosin (H&E) staining. These samples underwent dissection, paraffin block

embedding, and staining. All histology and cytology slides were examined using the same histopathology approach to avoid reading bias. Chronic granulomatous inflammation, acute necrotizing or suppurative inflammation, or even severe caseous necrosis are histological characteristics that are associated with lymphadenitis tuberculosis. Suggestive histopathology was the composite reference standard (CRS) employed for comparison. Through the addition, the H & E staining was carried out.

III. RESULTS

Giant cells and caseating granulomas make up the extrapulmonary TB histopathology with the central caseating necrosis. At the outpatient department (OPD), JSS Hospital Mysuru noted numerous cases of EPTB with enlarged lymphadenitis. The JSS Hospital in Mysuru's Department of Pathology is where the samples were obtained. Patients signed an informed consent form, and their demographic and historical information was obtained. The pathogen Mycobacterium tuberculosis was identified using direct microscopic stain, such as Hematoxylin and Eosin (H & E) and Acid Fats Bacilli (AFB), on biopsy samples that were Paraffin-Embedded Tissue samples.

The H & E staining was applied to the obtained blocks. One of the significantly more popular staining methods utilised by the histopathology investigation to evaluate the cellular and tissue structural detail is the H & E staining. To identify the stained tissues or lymph node metastases, aberrant cells with a nucleus larger than lymphocytes, deformation and deterioration of lymph sinuses, and erratic lymphatic structure were seen. Additionally, the staining method with minor changes determined by histopathology study. Here, the stain shows a a variety of cytoplasmic, nuclear, and extracellular matrix features, among other things. H & E staining technique has been the standard method for microscopic staining identification for many years and is still used today. All diagnostic groups' most prevalent presentation was cervical lymphadenopathy.(**Fig. 1**to**4**).

Histopathology biopsy samples that tested positive for MTB were stained with H&E and seen under a microscope.:

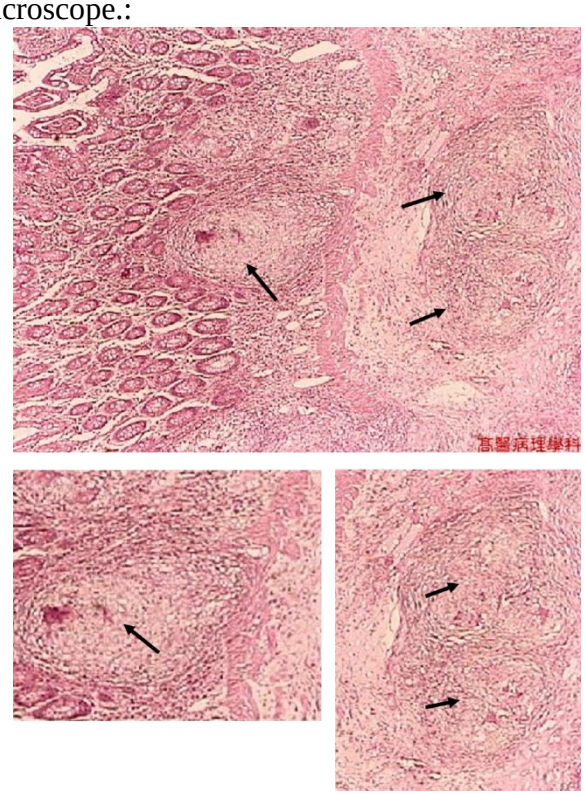


Figure 1: Granuloma cessations of the TB intestine (A) enlarged view with 1:10 ratio (B)(i, ii).

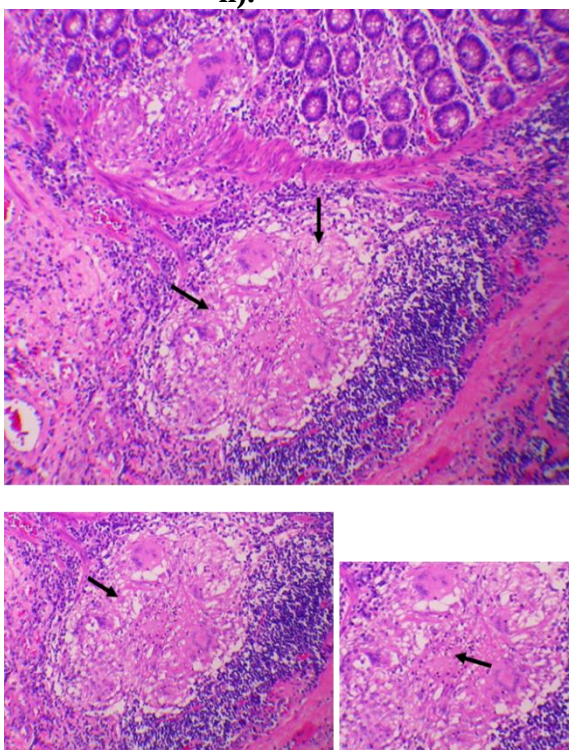
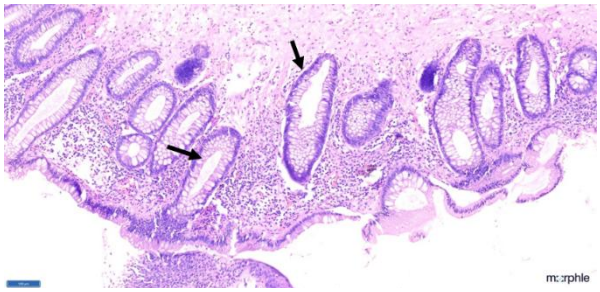
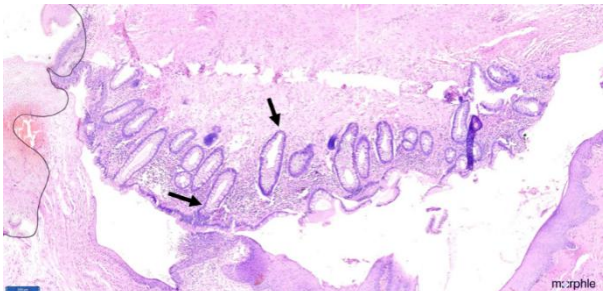


Figure 2: Granuloma cessations of the TB intestine (A) enlarged view with 1:10 ratio (B) (i, ii).



A) H&E x 400, (934)



B) H&E x20

Figure 3: TB with fissure abscessa lesion of Inflammatory bowel disease

MTB suspected cases' cytopathological analysis (n=250):

In order to understand how the pathogen spreads to lymph nodes, the positive histopathological tests were noted and the patient's X-ray data from the chest were reviewed. Of the 250 samples, 65 were determined to be normal and 185 to have EPTB. When the fine needle aspiration (FNA) revealed big cells, granulomas, necrosis with or without AFB, as well as caseous necrosis with or without granulation cells and AFB, the cytology was deemed positive (Table 1 & Figure 4).

	Normal Lesions	**Impression of EPTB**	**AFB Positive**	**AFB Negative**
EPTB Samples (n=250)	65	185	32	218

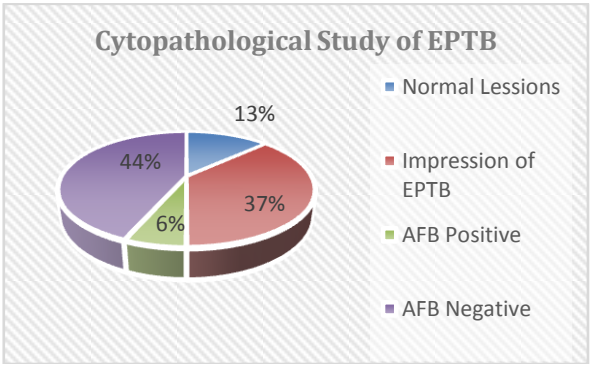


Figure 4: Cytopathological Study of EPTB

Each person's extra pulmonary tuberculosis symptoms are unique according on their immunity. It relies on how the infection is described to people with compromised immune systems. The most prevalent symptom was weight loss in 113 individuals, followed by fever in 56, anorexia in 35, night sweats in 46, and weight loss in 86 people. The cervical region (176 instances) was the most frequently affected by lymph node swelling, the submandibular (40 cases), supraclavicular (18 cases), multiple lymph node (8), axillary (6), inguinal node swelling (2), and other sites of infection (12 cases) (Figure 5).

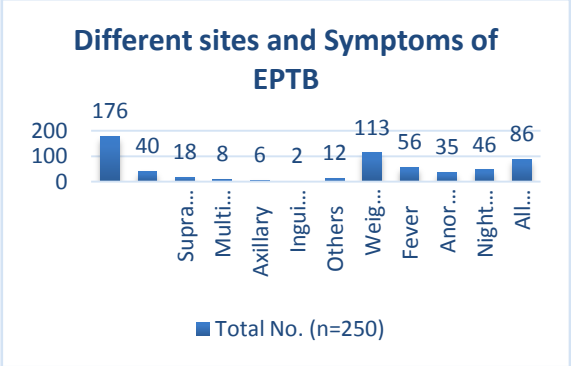


Figure 5: Different sites and Symptoms of EPTB

The gender distribution was more pronounced in men than in women. The younger age group of 21–30 was shown to be the most affected. Out of 250 total cases, 136 males and 114 females, or a 2:1 ratio, tested positive for EPTB. To determine the precise outcome in which the sample exhibits the most positives for MTB infection, several types of EPTB samples are treated to various procedures of infection. The molecular approach was shown to be infectious among all the methods, with the samples showing the most positive results for MTB in the PCR procedures. In order to obtain

a better and more meaningful result, the samples were next submitted to PCR following staining. The numerous techniques for detecting and identifying the EPTB infection by MTB are also included in the diagnosis (Table 2 and Figure 6).

Table 2: Various diagnostic techniques for finding MTB in the EPTB samples.

Different Procedure	**Cervical Lymph Nodes Biopsy (n = 150)**	**Abdominal biopsy (n = 21)**	**Other Right and Left Side Biopsy (79)**
	Case (n = 150) n (%)	Case (n = 21) n(%)	Case (n = 79) n(%)
H & E stain	**132**	**19**	**35**
Granuloma	**47**	**21**	**78**
Caseation	**78**	**05**	**42**
AFB Stain	**15**	**20**	**12**
PCR	**149**	**21**	**75**

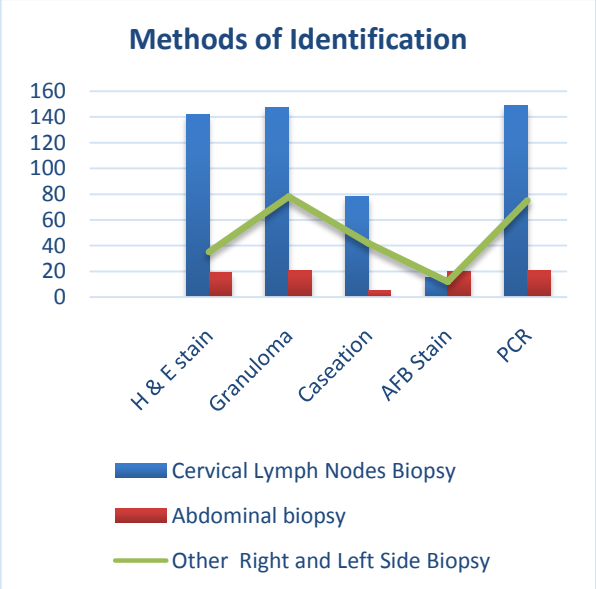


Figure 7: different diagnostic techniques for finding MTB in the EPTB samples

Difference in EPTB case results: There are various forms of EPTB, and some diseased organs exhibit variations in the cessation of granulomas. The majority of cases roughly 77 involve cervical lymph nodes, which are followed by the intestinal wall (73), the peritoneum (38), and the mesenteric lymph node (62) (Table 3 & Figure 8).

Table 3: Analysis of the variance in EPTB infection

Diagnosis (Cases)	**Cervical Lymph Nodes**	**Intestinal wall**	**Peritoneum**	**Mesenteric Lymph Nodes**	**Total number of specimens**
Reactive/Non Specific inflammation	37	15	6	10	68
Foreign body granuloma	26	32	16	42	116
Fungal granuloma	3	5	2	0	10
Parasitic granuloma	0	1	2	1	4
Malignancy	11	20	12	9	52
TOTAL	77	73	38	62	

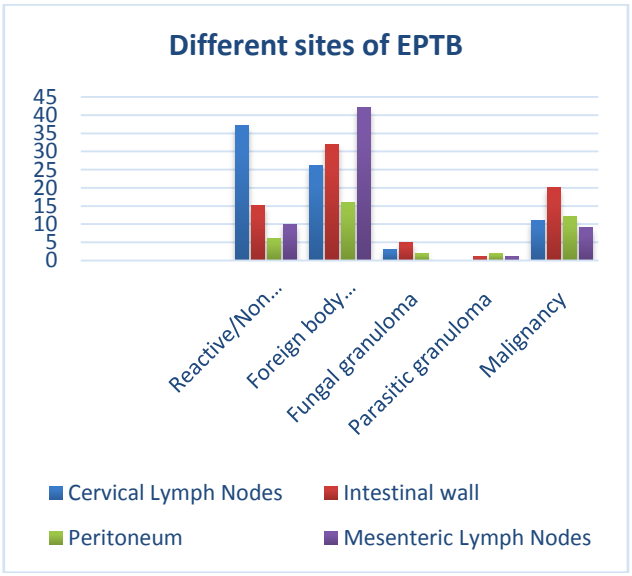


Figure 8: The variances in infection at the various EPTB analysis sites.

IV. DISCUSSION

The H & E staining method was used in the study's research findings to more accurately identify MTB at the primary stage. The stage of infection may vary according to the different types of identification methods, such as Granuloma & cessations, H & E staining, Z N staining, Molecular analysis, etc.,

In addition to still being an endemic disease, tuberculosis is the ninth most frequent cause of mortality from a single infectious component worldwide. Only 15% of tuberculosis cases prior to the HIV/AIDS epidemic were extrapulmonary, with pulmonary illnesses accounting for 85% of all cases. Extra pulmonary tuberculosis (EPTB) is the isolated presence of tuberculosis in any organ of the body other than the lungs. More than 50% of tuberculosis diagnoses among HIV-positive people and people with other co-morbid diseases are attributed to the EPTB, according to Mithila et al. (2016) [51].Every sample was collected in Mysore's JSS Hospital. 185 samples of H&E staining were used for the 250 paraffin-embedded, formalin-fixed tissue samples.
Some studies have showed the positive for EPTB out of 123 cases, 50% showed the positive for the AFB staining along with their load of bacteria content [17, 18, 19]. Other earlier studies also observed on the staining techniques for the necrosis and granuloma from the Das et al., [20] they have also observed the necrosis cessations infiltration with or without granulomas presences. In Kumar et al., studies they expressed more AFB positive when compared with the ZN staining [21]. It also showed the 47% of smear positivity for the epithelioid cells, and also explained that no difference between the ZN staining and fluorescent staining, higher percentage of the staining were showed positive is AFB around 93% and 88% were shown positive for MTB through the culture plates by Arora et al., (1990) [19]. Metre et al., (1987) [22] study also showed similar to the Arora et al., study, they found 64% for AFB and 60 for culturing positive for the MTB. From Radhika's et al., studies the RAB was shown positivity of about 26% and granuloma filtration was observed higher than 17.8% [23].Bacillary counting and AFB scoring have been used in some experiments to assess load of bacteria on fine needle aspiration smears relating to tuberculosis cytomorphological tendencies [23]. The old and practiced techniques are accurate but tedious, it takes more than 3-5days that is time consuming, and that technique cannot be practiced for the fast and reliable results. All 14 cases showed the presences of necrosis and from that 10 (71%) of the them showed neutrophilic infiltration. Immunosuppression is a possibility due to the patient's purulent inflammation and high bacterial load.

Other studies like Finfer et al., [24] showed the 31AFB positive out of 41cases, that gave 90%. In that 40% were from the HIV patients and 10% were from the non-HIV patients.In their cases, no traces of the co-infection disease like AIDS/HIV along with their risk factors. It has been demonstrated that fluorescent staining outperforms ZN staining in tissue samples, sputum smears, and fine needle aspirates. The utility of one over the other smear on fine needle aspiration along with the bacterial load of the cytomorphological techniques was never used for the detection of MTB hence the new techniques should be developed [24].With low bacterial load presence, fluorescent staining outperformed ZN staining along with H & E Staining for the identification. Some of the staining technique’s gives a good positive result for the MTB due to the high bacterial load content in the samples. Somlo et al. (1969) found that both staining methods were similar in specimens with several bacilli, but fluorescent method was larger than ZN stain along with few bacteria in sputum specimens were noticed [25].

Similar study from the Goel et al., [26] Since the development of granulomas and the appearance of the classic histopathological MTB image will be late occurrence, early MTB lesions cannot be identified by the histopathology technique, this also suggest as that this maybe transition between the growth and reproduction of the MTB bacteria for causing the disease.When histological analysis fails to provide a diagnosis, their IHC approach may be able to help in diagnosing the tuberculosis

in some of the cases. In tuberculosis-endemic countries, the tuberculosis prevalence was high and combined with parasitic infection are established [26]. One of their major technique in controlling the TB with IHC and PCR gave a positive result.

According to Mustafa et al. (2006), it is challenging to comprehend the good results of both tests on Norwegian foreign body granulomas. [27]. In endemic nations, the majority of granulomatous lesions without necrosis are believed to represent TB, even if this may not always be the case. One of the most effective techniques for diagnosing tuberculosis is histopathology, however there is one disadvantage of this technique is that it does not differentiate between the pathogenic MTB and Non-pathogenic MTB or other granulomatous diseases. Some research, such as Purohit et al., 2007 [28] showed a comparison between nested PCR and MTB detection. In published series where PCR was compared with the gold standard culture technique, results ranged from 60 to 90%, demonstrating the sensitivity and specificity of PCR for tuberculosis diagnosis [29, 30]. In addition, purohit et al., findings revealed a clear connection between culture and PCR, here one this is noticed all culture positive samples are noticed positive for the PCR technique too. Although PCR is increasingly utilised to detect mycobacteria from tissue samples, its use is constrained in nations like India due to the high cost of the equipment and reagents, contamination sensitivity, and technological demand [28, 30].

Immunostaining is more technically demanding and expensive than ZN staining, but the drawbacks are outweighed by the speed,sensitivity, and greater number of samples can be detected at lower level of objective without using oil immersion by microscopic method for the detection of the MTB.In addition, as compared to culture and H & E staining technique the immunostaining is considerably quicker since the findings are available within 1-2 days [28, 31].This technique shows the more specificityand sensitivity towards the detection of the MTB, when compared with nested PCR. But the technique is bit susceptible for the contamination and there are chances of contamination in the samples and MTB detection. Some research studies also explain that TB diagnostic should be improved for the low- and lower-income countries for the faster detection and diagnosis of the disease. Through the use of advanced technologies likeXpert MTB/RIF, automated molecular assay for accurate diagnostic assays, a third of the latest EPTB cases are unaccounted. This assay could be feasible and should be tested at different centres, as the current WHO research goals seek to recognise reasonsmainly improve the new diagnostic methods and technologies for the better identification of MTB [43, 44, 45, 46].In the Purohit et al.,(2007) [28] study they proved that there is some negative prediction from the immunostaining of anti-MPT64 and also helps to exclude the granulomatous lesions without necrosis.

Despite a general drop in TB warnings, extrapulmonary tuberculosis remained a serious medical problem in Australia. According to the study by Dominice et al., 2009 [47] and their series, 75% of patients were born outside of Australia, recognising immigrants as a higher-risk group for TB. In this study, 24% of patients more frequently associated with meningeal illness were identified solely on the basis of clinical factors, 20% of cases had positive histopathological and culture results. 50% of the samples showed rapid acid bacilli, Ziehl-Neelsen specimen staining remains a helpful, quick diagnostic procedure in series for TB. Drug resistance is still an issue in South-East Asians, where one in five people have one or more drug-resistant species of Mycobacterium tuberculosis. antibiotic resistance was only 7% prevalent overall, while 46% of isolates from refugees and 16% of South-East Asians were resistant to at least one antibiotic [48, 49]

The gender-wise distribution of the 250 cases in the EPTB samples was more pronounced in men than in women. The younger group of people aged 21 to 30 was shown to be the most infected age group. Out of 250 cases, 136 males and 114 females tested positive for EPTB, resulting in a ratio of 1.15:1. Similar to the Chandir et al., (2010) study, [50] reported the highest number of EPTB cases in Pakistan among people aged 15 to 29. In

contrast to Chandir et al.'s study, the female to male ratio in the Bisht et al. study was 3:1. It can be ascribed to the fact that Pakistani women suffer from vitamin D insufficiency, which has been noted as one of the risk factors for tuberculosis. Numerous studies have been conducted to determine how socioeconomic and cultural factors affect gender differences in tuberculosis. It has been discovered that a number of variables, including diet, educational level, adherence to treatment, and stigma related to tuberculosis, play a significant influence. According to Mithila et al. (2016), EPTB showed a little bias for females (1:1.05) but was otherwise almost similar in both sexes. Additionally, clamied that cases impacted younger age groups more frequently, accounting for 40.9% of all cases. The same study also discovered cases of EPTB at uncommon locations such the scrotum, vulva, eyelid, oral vestibule, and wrist [51].

Out of 250 individuals, the cervical region had the highest lymph node swelling (176 instances), followed by the submandibular (40 cases), supraclavicular (18 cases), numerous lymph nodes (8 cases), axillary (6 cases), inguinal node swelling (2 cases), and other sites of infection (12 cases). In a way similar to this, cutaneous tuberculosis (24.27%) was the most often affected location in the distribution of sites implicated in EPTB, followed by the lymph nodes (19.42%) and the skeletal system (16.50%). In a research by Mithila et al., cases were found in the vulva, eyelid, oral vestibule, wrist, and scrotum, among other locations. Genital TB was only discovered in 0.2% to 2% of all gynaecological cases, and only 1-2 percent of those cases included the external genitalia [51]. Fallopian tubes and endometrium become infected as a result of blood dissemination, direct spread, or sexual encounter with someone who has epididymal or renal tuberculosis. Except in endemic areas, genitourinary tuberculosis accounts for 20–73% of all cases of EPTB and is extremely uncommon in children's populations [52]. In prior research, blepharoplasty-related eye lid tuberculosis was reported [53].

Women and 21-30 age group, then 11-20 year, may draw the conclusion that the analysis is independent of modifiable risk factors for EPTB, according to Mithila et al.'s [51] research. Definitive tuberculosis diagnosis requires the use of histopathological, microbiological, cytopathological and molecular approaches to explain *M. tuberculosis*. Histopathology becomes more important when EPTB has enigmatic occult spots. One of the best ways to diagnose EPTB is in particular when combined with tissue culture and ZN staining. Molecular diagnostic methods are frequently employed for a rapid and accurate tuberculosis diagnosis [51].

Extrapulmonary tuberculosis is difficult to diagnose for several reasons: a scarcity of sufficient sample quantities or volumes the other different methods of identification of MTB that includes histology/cytology, culturing, staining techniques along with histopathology staining techniques, nucleic acid amplification-based techniques, PCR etc., these results in the non-uniform distribution of the bacteria and some widely available technique used for processing for all the forms of extrapulmonary samples.

A larger multicentre study such as H & E staining and PCR with more specimens could help determine MTB, this technique can be used as the stand-alone test for EPTB cases instead of the ZN stain test.Since traditional microbiological methods perform poorly in extrapulmonary specimens, PCR tests are being used more frequently in tuberculosis laboratory diagnosis.The PCR assay for the detection of the MTB can be made as one of the important techniques in high-prevalence countries and regions so that it leads to the control of TB and especially with controlling of EPTB. In today's practise, physicians do not initiate or discontinue care for this disorder alone based on PCR performance. We compared the test results to histology, cytology, and microbiology along with systematic clinical follow-up of the patient’s history and their demographic data, by all the aspects the AFB and H & E staining are practiced for the detection of the EPTB along with its specimens. Hence, current study mainly focused for the H & E staining techniques and the followed by the PCR method. Where H & E gives 75% accuracy when compared with Molecular methods, such as PCR gives the99% accuracy for the detection of the MTB in the EPTB samples.

V. CONCULSION

To, summarize that EPTB is increasing its sites of infection and creating a dilemma for the doctors to prescribe the drugs. Hence there is urgent need to understand the types of EPTB and their different sites where it is infecting other rare sites of the body too. In India, EPTB should be taken care in part of diagnosis. Thehistopathological, microbiological, cytopathological and molecular methods helps in diagnosis of EPTB. When it comes to mysterious occult places, histopathology aids in the identification of the EPTB. Using biopsy tissue samples and ZN staining to check for EPTB is another important diagnostic technique. Tuberculosis can also be accurately and extensively detected using molecular diagnostic techniques.

Current study showed the males and younger age group of 31-40 years due to their living habit and their Socio-economic status. Nowadays EPTB is also becoming an issue in the countries in respect to pulmonary. Though there are different types of EPTB but majorly we could see mycobacterium affects the Lymph node which is most seen EPTB in patients.The *Mycobacterium tuberculosis* which cannot be identified through the H& E staining method they are identified by Molecular diagnosis method. So, it's more important to researcher to carry the work regarding the EPTB to cure the diseases as early as possible. In the end, this would lead to a general decrease in EPTB morbidity and mortality. ZN is preferred over fluorescent staining when there is a low bacterial burden. ZN and H & E stain, on the other hand, can be incredibly helpful in particular cases if a fluorescent microscope are not utilised.

FUNDING ACKNOWLEDGEMENT

We acknowledge the resources and financial support for the study, provided by the Indian Council of Medical Research (ICMR) New Delhi, the Order/File No: Fellowship/TB/38/2019/ECD-1.

AUTHORS CONTRIBUTION STATEMENT

Dr. Sumana K was initiated for this research articles the manuscript. Ms. TalluriRameshwari K R has finalized the topic and written the original Manuscript, Dr Nazia Khan & Dr Ritik Kashwani suggested ideas for this research paper, and also helped in writing the manuscript order wise, Vikas Malgotra and Arun Shanmugam helped along with flow chart and diagrams, reviewed and edited the manuscript. etc. and Finally, all authors have discussed the methodology, results and contributed to the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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