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To Assess the Burden of and Trends in Extra Pulmonary Tuberculosis at a Tertiary Care Hospital in India

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Authors' contributions

This work was carried out in collaboration between all authors. Author SF designed the study, wrote the protocol, did the literature searches, analyses of the study and wrote the first draft of the manuscript. Author NMA managed the clinical diagnosis, referral of samples to the laboratories and treatment of the cases and author FF managed the cytological and histopathological workup of the specimens. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Extra pulmonary form of tuberculosis is an important public health disease which cannot be ignored because of its low transmissibility. Data on the exact burden of the disease in developing countries is scarce.
Aim: To assess the burden of the disease in a tertiary care hospital of India. To study the clinical trends in the disease, and the utility of various diagnostic modalities for its diagnosis. To identify the Mycobacterial species and perform drug susceptibility test.
Materials and Methods: A cross sectional study was carried out for a period of two years. A total of one hundred and forty seven samples were tested for extrapulmonary tuberculosis using a combination of bacteriological, cytological, histological and biochemical techniques to achieve proper diagnosis of the disease.
Results: Young adults and females predominated in the study group and positive cases.

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Microbiologically, 26% of the specimens were positive. Eighteen percent of them were found to be culture positive for *M. tuberculosis*. Smear by Ziehl Neelsen stain was positive in 9%. A combination of culture media both solid and liquid maximized the yield of Mycobacteria. Lymph node tuberculosis was found to be the predominant type followed by others. Fifteen percent of the strains were found to be resistant to the first line drugs used in treatment of tuberculosis. Cytology and biochemical findings were found to be less specific in diagnosis of extrapulmonary tuberculosis.

Conclusion: Extrapulmonary form of tuberculosis is seen in significant number of the suspects. Hence, attention should be paid towards its proper and early diagnosis followed by rational management, as if neglected may lead to associated complications and sequalae.

Keywords: Extrapulmonary tuberculosis; Mycobacterium tuberculosis complex; Mycobacteria growth indicator tube; Lowenstein Jensen media; microscopy; cytology.

1. BACKGROUND

Consumption aka tuberculosis is an age old disease. From time immemorial, right from the Egyptian mummies till today it remains a major public health problem. As per the World Health organization [WHO] report, it is the seventh leading cause of death worldwide [1]. Globally, around 8.8 million people develop tuberculosis annually and 1.45 million die of tuberculosis [2]. The major brunt of the disease is borne by the developing nations, with Asia having the maximum percent [55%] of the affected individuals followed by the sub-Saharan Africa [37%] [3]. Because of its high prevalence in lower socio economic strata of the community it is also called as the disease of the poor. It mainly involves lungs but can virtually affect any organ in the body. Extrapulmonary tuberculosis [EPTB] under Revised National Tuberculosis Control Programme [RNTCP] in India is defined as tuberculosis of any organ other than the lungs which includes pleura, lymph nodes, gastrointestinal, bone and joints, meningeal, genitourinary, cutaneous and tuberculoma of the brain [4]. Cases with primary pulmonary and peripheral lymph node involvement are considered as cases of pulmonary tuberculosis only [5]. Overall EPTB accounts for one third of all the cases of tuberculosis [6]. The pulmonary form of the disease is the commonest and easy to diagnose. Whereas, diagnosis of extrapulmonary form of the disease is challenging and often remains undiagnosed and untreated. Diagnosis of EPTB is difficult for reasons like the paucibacillary nature of the disease and difficulty in collecting the right sample, which cannot be repeated. Diagnosis of EPTB by conventional methods using Ziehl Neelsen stain and culture on solid media is time consuming and has got poor sensitivity. Cytological and histopathological findings with predominant lymphocytosis and granuloma formation can be seen in many chronic infections and sarcoidosis as well. Hence it is not always conclusive of tuberculsosis [7]. Therefore, finding of acid fast bacilli in direct smear and culture remains the gold standard. In order to improve the detection of Mycobacteria and reduce the turnaround time for diagnosis of tuberculosis a wide variety of automated systems are available in market; with reported sensitivity and specificity. Among them, Mycobacteria growth indicator tube [MGIT] 960 and MGIT 320 approved by WHO appears to be more suitable and cost effective method [8–11]. Use of Polymerase chain reaction [PCR] for diagnosis of tuberculosis is more sensitive with the shortest turnaround time of less than one day. However, it has own pitfalls like requirement of infrastructure for set up of a molecular laboratory, need of technical expertise etc. Further, the presence of PCR inhibitors in the sample gives false negative results reducing its sensitivity [12-15]. Moreover, the test cannot distinguish between active and latent disease. Therefore, in order to determine the prevalence of the disease the ideal diagnostic test appears to be isolation of the bacterium by culture or detection of bacteria by microscopy. Reports on EPTB from India are very few. To know the exact burden of the EPTB disease in developing countries, its clinical presentation and efficacy of various diagnostics modalities further studies are required.

2. AIM

To estimate the burden of EPTB in clinically suspected cases attending a tertiary care hospital over a period of two years from Jan. 2013- Jan. 2015. To study the various clinical types of EPTB prevalent. To evaluate the efficacy of various diagnostic methods-bacteriological, cytopathology and biochemical test in its diagnosis. To identify the Mycobacterial

species involved as *M. tuberculosis* complex [M.tb complex]. And to carry out drug susceptibility test for it.

3. MATERIALS AND METHODS

A cross sectional study on EPTB suspects was conducted at Princess Esra hospital a teaching hospital of Deccan College of Medical Sciences from January 2013 to January 2015. Case inclusion criteria were as per the WHO guidelines for EPTB [16]. A total of 147 cases with clinical suspicion of EPTB were referred from various specialties to microbiology laboratory for diagnosis. Clinical specimens were collected aseptically by an experienced specialist through fine needle aspiration [FNA], pleural tap, excision biopsy and computerized tomography guided aspiration based on the site involved. Microbiological analysis was done using direct microscopy by ZN stain and culture. Isolation of Mycobacteria from various clinical samples was attempted using a combination of two different culture methods. Namely liquid culture media i.e Midlle Brook 7H9 broth for automated TB culture system [MGIT320] from Beckton and Dickinson USA and Lowenstein Jenson media [LJ] from Hi Media laboratories. M. tb complex species confirmation was achieved by testing the positive isolates of Mycobacteria for M. tuberculosis antigen test; using the commercially available Mycobacterial protein antigen i.e. MPT 64 antigen test provided by Standard Diagnostics Bioloine Ltd. Germany which is specific for M. tb complex. Drug susceptibility test for first line anti tuberculosis drugs [ATT] was carried out using readymade kit called SIRE from BD.USA.

3.1 Specimen Processing for Mycobacterial Culture and Smear

All the clinical specimens were processed by the standard N-acetyl-L-cysteine sodium hydroxide digestion-decontamination technique for inoculation into MGIT tubes and LJ Medium. The specimens were then utilized for ZN staining. [17,18].

3.2 Quality Control

Was achieved by following the manufacturer instruction from BD MGIT 320 guidelines and test was carried out using the standard strains of M. tb complex H37 RV and ATCC strains.

3.3 Collection of Specimen for Cytology

Fine needle aspiration was performed under aseptic precautions using 18-21 G. needle and slides were fixed in alcohol, followed by Haemotoxylin and Eosin [H & E] staining and microscopy. Other cytological material were processed and stained with H & E followed by microscopy. Body fluids cytology was taken to be suggestive of tuberculosis when it was exudative with protein >3 gm% and predominant lymphocytes. FNA cytology of tuberculosis was evidenced by predominant lymphocytosis, necrosis, hypocellularity, and epitheloid granuloma with or without multinucleated giant cells with and without acid fast bacilli.

3.4 Biochemical Analysis

Various body fluids were analysed biochemically for proteins, sugar, Lactate Dehydrogenase [LDH] and Adenosine deaminase [ADA] levels. Biochemical findings were suggestive of tuberculosis when protein was >3 gm%, sugar less than two thirds of the blood levels, ADA with cut off > 20 IU/L and LDH > 130 IU/L.

4. RESULTS

4.1 Bacteriological Findings

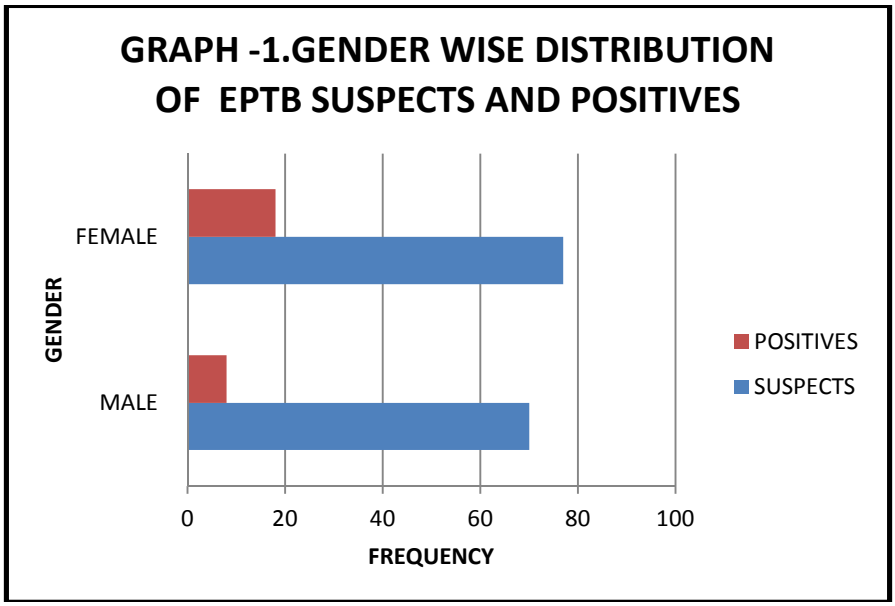
Of the 147 extrapulmonary tuberculosis suspects analysed bacteriologically using microscopy and culture as various methods for diagnosis, thirty nine 26% of the samples were found to be positive bacteriologically i.e. by either direct microscopy, culture or by both smear and culture. Smear alone was positive in 13 [9%] of the specimens, culture alone was found to be positive in 14 [9.5%] and culture and smear both were positive for 12 [8%] specimens. Overall, culture positivity was around 18%. In the present study MGIT 320 system and LJ media together could detect 6 of the 26 or 23% isolates; LJ media alone could detect 2/26 or 8%. MGIT alone could detect 18/26 or 69% of the isolates. Of the 26 strains of M.tb complex isolated and confirmed by MPT64 antigen test. Four strains were showing resistance to first line anti–tuberculosis drugs. The rest of the strains were pan susceptible.

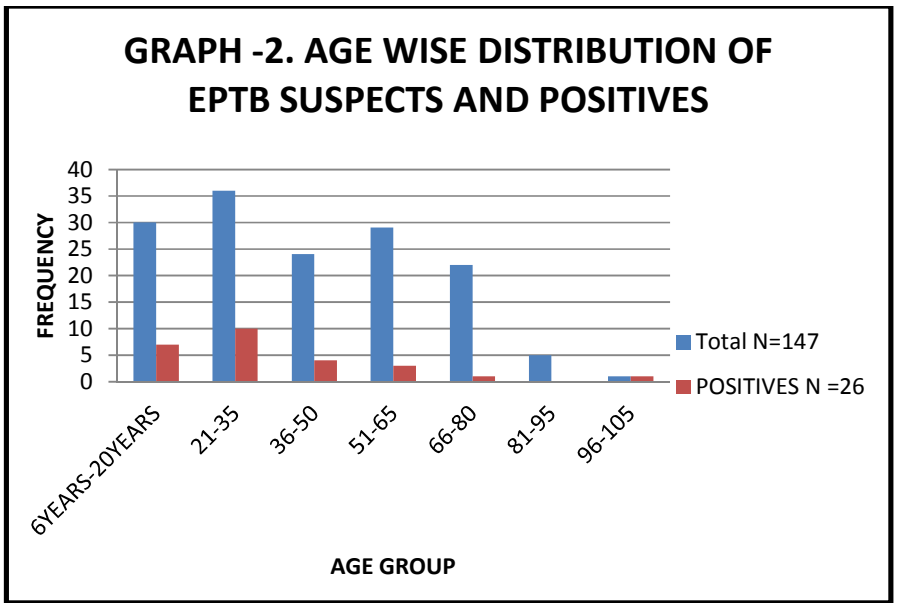
No significant variation in the gender ratio was observed in the number of samples submitted for processing. Around 77/147 or 52% were females and 70/147 or 48% were males. Anyhow female's outnumbered males in laboratory confirmed extra pulmonary tuberculosis cases; a difference of 2.25 in gender ratio was noticed. Females being 18/26 [69%] and males 8/26 [31%] as shown in Graph 1. The mean age for

males in suspects was 46.11±23.46 and for females 39.42±27.74 .In the laboratory confirmed cases mean age for females was 35±22.77 and for males 39.75±16.83 respectively.

It is noted that young adults less than thirty five years of age accounted for the majority of the suspects 66/147 [45%] and of culture positive cases 21/26 [73%] as shown in Graph 2. Therefore, it is evident that the disease is more prevalent in the economically active and reproductive age group of the society.

The majority of the samples received for test were pleural effusion accounting for 72/147 [49%] followed by peripheral lymph nodes accounting for 39/147 [27%], gastrointestinal accounting for 17/147 [12%], osteo articular accounting for 11/147 [7%], pus accounting for 5/147 [3%] and genitourinary in 3/147 [2%], respectively. In the present study, lymph node tuberculosis was the predominant type having 12/26 [46%] of the positive cases followed by pleural effusion in 5/26 [19%], osteo articular in 4/26 [15%], pus in 2/26 [8%], gastrointestinal in 2/26 [8%] and genito urinary in 1/26 [4%].





4.2 Results of Biochemical Analysis of the Body Fluids

In the present study, biochemical analysis of 89 body fluids in the form of pleural, peritoneal/ ascitic fluid showed that proteins were >2 gm% in 50%, none were having protein >3 gm%. Sugar was <2/3 of blood sugar in 100% of the cases and ADA & LDH were raised significantly in all i.e. 100% of the cases. Microbiologically only 26% of the specimens were positive for tuberculosis.

4.3 Cytology Results

Of the total 147 specimen submitted for cytological diagnosis of extra pulmonary tuberculosis in pathology laboratory during the study period, seventy two were pleural fluids, thirty nine lymph node material obtained by FNA, seventeen were peritoneal fluids, five were pus samples, eleven were osteo articular and three genitourinary. Cytology was suggestive of tuberculosis in 99 [67.34%]. Twenty percent of the specimens were positive by both cytology and culture. And smear and cytology was positive for 21 [21%]. The remaining 58 [59%] were only cytology positive.

5. DISCUSSION

The prevalence of extrapulmonary tuberculosis in non HIV cases ranges between 15-20% of all the tuberculosis cases as reported by various authors [19-24]. In HIV patients it accounts for a greater percentage of nearly 50-70% [24,25]. Off late worldwide EPTB is on rise both in non HIV & the HIV patients, though variations have been observed in its prevalence from country to country. In the present study involving non HIV host the number of bacteriologically positive cases is 26% and culture confirmed 18% which is similar to the one reported by other authors [26-35]. According to the literature, diagnosis of extrapulmonary tuberculosis is difficult and depends on factors like the quality of the sample received which cannot be repeated and the methods adopted for its isolation. The isolation rates reported so far are variable. Further, it is noticed that a combination of methods provides a better yield of Mycobacteria than relying on a single method; even WHO has emphasized its significance in its guidelines.

Moreover, further discrepancies observed in isolation rates could be explained by considering the facts like the choice of media, processing methods, the study group whether it is purely extrapulmonary or both pulmonary and extra pulmonary [36-39].

The disease continues to be more prevalent in females. In the present study it is seen in 69% of the laboratory confirmed cases, for reasons not yet established and requires further workup. Similar findings were also noticed by other authors [7,33,36,40]. Young people in the age group 16-35 years formed the predominant subgroup among both the suspects and the EPTB confirmed cases as reported by other authors [41,42]. Lymph node tuberculosis still remains the predominant type with a percentage positivity of 46%. Pleural fluid [19%] was the next common type, followed by osteo articular then abdominal and pus and least the genitourinary type as reported by other authors [33,37,38,43-47]. Diagnosis of EPTB by isolation of Mycobacteria in the clinical specimen remains the gold standard technique but has got its own limitations as mentioned earlier in the previous paragraph. In the present study isolation of, Mycobacteria was done using two different methods, liquid and solid media. Culture alone was found to be positive in 9.5% of the cases which is similar to the one reported by Hillemann D et al. [26] and 8% of the samples were positive by both culture and smear.

Here the use of the automated MGIT 320 system from BD looks to be more promising with the minimal turnaround time for smear positive samples as 5-9 days and for smear negative15-36 days [19-20,28,46-48]. Definitely these automated systems play a remarkable role in early diagnosis of the disease and in no time will also provide relevant information on selection of the anti- tuberculous drugs for treatment. In the present study, MGIT 320 system alone could detect 69% of the isolates. Hence is superior to the conventional LJ media which alone could detect 8% isolates. A combination of the two methods helped in isolating 23% of isolates [28,49]. Therefore, use of combination media works well, even though the sample size of missed ones by MGIT is insignificant, but for a disease like tuberculosis it matters. In extrapulmonary tuberculosis microscopy results are not so encouraging for reasons like pauci bacillary nature of the disease and the quality of the sample received. In the present study microscopy alone could detect only 9% of the cases which is similar to the results reported by others [26,29,46,50,51]. M.tb complex has been isolated from 93% of culture positive isolates.

One of the reasons for this could be due to the fact that the study included non HIV cases as non tuberculous mycobacteria are more common in the immunocompromized host. Moreover, *M. tuberculosis* is endemic in developing nations and can remain latent to get reactivated and cause EPTB at any time in life [47]. Resistance to the first line anti - tuberculosis drugs was detected in 15% of the cases in the present study as reported by Hillemann D et al., S.K. Sharma et al. and WHO surveillance report on resistance to anti-tuberculsis drugs. The results of biochemical findings proved to be non specific and inconclusive. Cytology was suggestive of tuberculosis in 67.34% which is similar to the reports by Y. Zenebe et al. [50]. Twenty percent of the samples were positive by both cytology and culture. Attributing significance to cytology and biochemical findings may lead to over diagnosis and in judicious use of anti tuberculosis drugs which contributes to drug resistance in *M. tuberculosis* [52,53].

6. CONCLUSION

Extra pulmonary tuberculosis is seen in a substantial number of the suspects. Disease was found to be more common in young adults and females. Peripheral lymph node tuberculosis was the predominant type. A combination of diagnostic methods especially direct microscopy and culture by use of liquid and solid media helps in enhancing the isolation rates and confirming the etiology. Rapid identification test for M.tb. complex like MPT64 antigen detection test finds place for confirming the type species involved with good sensitivity and specificity. It is wiser to read cytology and biochemical findings in light of bacteriological results as alone they are less specific. Resistance is emerging to anti tuberculosis drugs even in extra pulmonary form of disease. Hence the use of anti tuberculosis drugs has to be justified as per the laboratory results of drug susceptibility pattern. The authors deeply thank the management of Deccan College of medical sciences, Princess Esra Hospital and the technical staff of all the central laboratories for providing timely help.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Prior to the study ethical clearance was obtained from the ethical committee of the institute.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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