Background: Tuberculosis is a major global health problem. Tuberculous lymphadenopathy is a most common form of extrapulmonary tuberculosis (EPTB), constitutes 35% of all cases of EPTB. Due to the paucibacillary nature of specimens, smear microscopy and culture offer low sensitivity. Methods: The aim of the present study was to find the clinicodemographic profiles and comparing the performance of Xpert MTB/RIF, conventional polymerase chain reaction (PCR), mycobacteria growth indicator tube (MGIT) 960, histopathological examination, and clinical follow-up of patients in diagnosing of smear-negative tuberculous lymphadenopathy. Results: A total of 140 clinically suspected cervical tuberculous lymphadenitis cases were enrolled in this study. MGIT-960 culture, conventional PCR, and Xpert MTB/RIF were performed. Most of the patients presented with unilateral (87.14%), single (81.42%), matted (87.85%) lymph nodes, 3 cm–6 cm (52.14%), commonly in the right side (68.02%), and associated lung lesion was found in 12.86% of cases. The detection rates of Mycobacterium tuberculosis complex (MTBC) by Xpert MTB/RIF, conventional PCR, and MGIT were 25.71%, 20.71%, and 17.85%, respectively. Both the tests: Xpert MTB/RIF and PCR, PCR and MGIT, Xpert MTB/RIF and MGIT were positive in 15.71%, 15.71%, and 11.42% of cases, respectively. Most of the patients (74.1%) were cured with 6 months of antitubercular drugs. Conclusion: Clinicians often face the diagnostic dilemmas presented in the study. Individual modalities of the diagnosis are available, but all have drawbacks with varied sensitivity and specificity. Combining the available clinical, radiological, and microbiological modality to reach early diagnosis can go a long way to avoid misdiagnosis and unnecessary delay in treatment, especially in cases, without the pulmonary involvement and fulfilling the aim of National Tuberculosis Control Programme for EPTB cases.

Keywords: Extrapulmonary tuberculosis, lymphadenitis, tuberculosis

Introduction

Tuberculosis (TB) is a major public health problem and a significant cause of morbidity and mortality in developing countries.[1,2] Nearly 9.6 million people get infected, and 1.5 million die from tuberculosis globally every year.[3] Extrapulmonary tuberculosis (EPTB) is seen in nearly 15%–20% of all cases of TB.[4] Tuberculous lymphadenopathy is the most common form of EPTB, constitutes 35% of all cases of EPTB.[4–7] Cervical lymph nodes are the most common site of tuberculous lymphadenopathy in 60%–90% of cases, and its diagnosis remains a challenge.[4] Due to the paucibacillary nature of specimens, smear microscopy and culture offer low sensitivity.[1,8] Smear microscopy has been found to be positive in <10% of patients whereas cultures for mycobacteria found to be positive in 39%–80% of cases.[1] Granulomatous lymphadenopathy and caseation-necrosis on histopathological examination may occur in diseases (sarcoidosis, fungal infections, carcinoma, and other inflammatory conditions) other than TB, therefore, may not be very useful.[1,9] Given the above, relying on a single mode of diagnosis will always have less sensitivity with increased morbidity and mortality. If such an EPTB case has concomitant pulmonary TB, it becomes a source of spread to the community. Thus, the use of conventional methods and histopathological examination in conjunction with molecular techniques is all to be done in conjunction to cover-up the low-sensitivity issue of individual tests and further help...
in the detection of *Mycobacterium tuberculosis* (MTB) in tuberculous lymphadenitis. The aim of the present study was to find the clinicodemographic profiles and comparing the performance of Xpert MTB/RIF, conventional polymerase chain reaction (PCR), mycobacteria growth indicator tube (MGIT) 960, histopathological examination, and clinical follow-up of patients in the diagnosis of smear-negative tuberculous lymphadenopathy.

**Methods**

This study was conducted at the Tuberculosis division, Department of Microbiology, All India Institute of Medical Sciences, New Delhi, between May 2015 and May 2016. The age of the patients ranged from 9 to 85 years of age. The patients with cervical lymphadenopathy were investigated for tubercular etiology. A total of 140 patients with enlargement of cervical lymph node or swelling of the neck or draining sinus presenting to different specialties of our hospital were studied in detail. As per the Revised National Tuberculosis Control Programme (RNTCP), fine-needle aspiration (FNA) was performed in all the patients after detailed clinical history and examination. Excision biopsy was done in case of negative and doubtful FNA cytology (FNAC) results. Erythrocyte sedimentation rate (ESR), tuberculin skin test, and chest radiograph were done in all the patients.

**Collection and processing of samples**

FNA/excision biopsy from 140 patients was received in TB division (Department of Microbiology, All India Institute of Medical Sciences, New Delhi) for smear examination Ziehl–Neelsen (ZN) staining, culture (MGIT 960 system), Xpert MTB/RIF, and PCR. Another part was subjected to histopathological examination. The samples were processed using the NALC-NaOH method (N-acetyl-L-cysteine-sodium citrate method) as per standard method for making smear, culture, Xpert MTB/RIF, and PCR tests.

**Smear examination**

After processing, the samples were subjected to smear examination using ZN staining method and examined under light microscope, and interpretation was done as per RNTCP guidelines.

**Mycobacteria growth indicator tube 960**

Decontaminated samples were inoculated into MGIT tube (containing 7H9 medium), MGIT 960 nonradiometric automated isolation system (Becton Dickinson, Sparks, MD, USA), and positive cultures were further confirmed using Tb identification test (TBcID, Becton Dickinson, Sparks, MD, USA).

**Xpert MTB/RIF**

The Xpert was performed as per manufacturer’s instructions (Cepheid, Sunnyvale, CA). In this assay, sample reagent was added into clinical samples at a 2:1 ratio and was incubated for 10 min. At room temperature, shaken again and kept for 5 min, then 3 ml of inactivated material was transferred to the cartridge. The cartridges were inserted into test platform and results were produced after 90 min. The interpretation of data from tests was software based and not user dependent.

**DNA isolation and polymerase chain reaction**

Sample and reagent preparation and PCR amplification were carried out in separate rooms using utmost precautions to minimize crossover and carryover contamination. DNA extraction was standardized by phenol chloroform isooamy alcohol method using the lysis buffer containing 20 mM Tris HCL, 0.5% Tween 20, and 1 mg/ml proteinase k for 16 h at 56°C. The DNA was amplified using primer sequences MPT1 (5'-TCC GCT GCC AGT CGT CTT CC-3') and MPT2 (5'-GTC CTC GCG AGT CTA GGC CA-3') in 50-µl reaction mixtures as per previously published protocol. These primers generate amplicons of 240 bp for MPT-64 gene for MTB. During each PCR, appropriate positive control (100 pg of H37Ra DNA) and negative controls (sterile distilled water) were run with the samples. The amplified products were analyzed by gel electrophoresis.

Composite reference standards (CRS) have been considered as gold standard in the present study to overcome such issues. CRS for aspirates and biopsy samples include the following: any two of culture/histopathology/radiological findings/response to treatment positive. Response to treatment was assessed regarding the improvement of signs and symptoms such as fever, weight gain, improvement in general well-being, and decrease in size of the lymph node.

**Statistical analysis**

Data were collected and analyzed using STATA/SE 14.0 statistical software (Stata Corp LLC, 4905 Lakeway Drive college station, Texas, USA). Categorical data were described using numbers and percentages. Our data were presented in the form of tables, and descriptive statistics were analyzed in percentages. P value was calculated using Chi-square test to analyze statistical significances.

**Results**

**Clinical and demographic characteristics of study population**

A total of 140 patients with cervical lymphadenopathy were included in the study. Among these 140 patients, 65 (46.42%) were male, and 75 (53.57%) were female; the male-to-female ratio was 1:1.15. The age of the patients ranged from 9 to 85 years with a mean age of 59.55 ± 14.46 years. Among these 140 patients, 123 (87.85%) patients presented with solid lymph node, 14 (10%) with abscess, and 3 (2.14%) with discharging sinus. Unilateral cervical lymph node involvement was seen in 122 (87.14%) cases, more in the right side 83 (68.02%) than 39 (31.96%) left side. Lymph node size of 3cm–6 cm in diameter was seen in 74 (52.85%) cases, followed by >6-cm diameter in 42 (30%), and <3-cm diameter in 24 (17.14%) cases, and associated lung lesion was found in 18 (12.86%)
by X-ray cases. There was no significant relationship between site of involvement and enlarged lymph node with gender of the patients. The systemic features, such as fever 105 (75%), weight loss 83 (59.28%), and night sweats 81 (57.85%), were found in patients [Table 1]. ESR was raised in 67 (47.85%) patients, and the Mantoux test was positive in 44 (31.42%) cases. All samples were negative by smear microscopy.

Performance of Xpert MTB/RIF, polymerase chain reaction, mycobacteria growth indicator tube 960, and fine-needle aspiration cytology for diagnosis of cervical tuberculous lymphadenitis

Among the 140 suspected cervical tuberculous lymphadenitis patients, detection rates of Mycobacterium tuberculosis complex (MTBC) by Xpert MTB/RIF, conventional PCR, and MGIT 960 were 25.71%, 20.71%, and 17.85%, respectively. Xpert MTB/RIF detected 16 cases, which were negative by MGIT960 while in five cases were missed by Xpert MTB/RIF which were positive by MGIT960. There were eight culture-positive cases, which were negative on PCR and 12 culture-negative cases were PCR positive. Xpert MTB/RIF and PCR, PCR and MGIT 960, and Xpert MTB/RIF and MGIT 960 were positive in 15.71%, 15.71%, and 11.42% cases, respectively [Table 2]. All microbiological tests (MGIT 960, Xpert MTB/RIF, and PCR) were positive in 11.42% of cases. Sensitivity and specificity of GeneXpert and PCR against gold standard are shown in Table 3.

Response to treatment

Category I treatment was started in 80 (57.14%) patients as per RNTCP guidelines and most of these patients (75%) responded within 6 months of treatment, 13.75% of patients cured within 9 months, and 11.25% needed 1 year of treatment. All the Xpert MTB/RIF positive cases were rifampicin sensitive.

There was no reduction in the size of the lymph node in six (4.29%) cases even after antitubercular treatment; however, there was an improvement in general well-being and weight gain. In these six patients, finally, surgery was done to remove the lymph nodes. About 7.85% (n = 11) of cases were negative by all the three microbiological (MGIT 960, Xpert MTB/RIF, and PCR) investigations as well as inconclusive FNAC results. The antitubercular treatment was started in these patients on the basis of clinical suspicion such as raised ESR, positive Mantoux test, and the size of the lymph node decreased in these 11 cases also within 6 months of the treatment.

DISCUSSION

TB is a major cause of morbidity and mortality worldwide. In India, tuberculous lymphadenopathy is one of the most common types of lymphadenopathy encountered.[17] Both tuberculous and nontuberculous mycobacteria can be responsible for cervical lymphadenitis and its diagnosis, and management is still a problem despite its increasing worldwide incidence.[18-20] TB is considered as the most common opportunistic infection in belts where HIV infection is rampant.[21]

Table 1: Clinical characteristics of cervical lymphadenitis cases

| Characteristics                  | n (%) | P     |
|---------------------------------|-------|-------|
| Site of involvement             |       |       |
| Unilateral                      | 122 (87.14) | 0.083 |
| Right                           | 95 (67.9)  |       |
| Left                            | 45 (32.14)  |       |
| Bilateral                       | 18 (12.85)  |       |
| Number of enlarged lymph node   |       |       |
| Single                          | 114 (81.42) | 0.193 |
| Multiple                        | 26 (18.57)  |       |
| Size of involved lymph node     |       |       |
| <3                              | 24 (17.14)  | 0.347 |
| 3-6                             | 74 (52.85)  |       |
| >6                              | 42 (30)     |       |
| Mode of presentation            |       |       |
| Solid                           | 123 (87.85) | 0.761 |
| Abscess                         | 14 (10)     |       |
| Discharging sinus               | 3 (2.14)    |       |

Table 2: Comparative analysis of composite reference standard, Xpert MTB/RIF, conventional polymerase chain reaction, and mycobacteria growth indicator tube for the diagnosis of cervical tuberculous lymphadenitis

| Xpert MTB/RIF* | MGIT\* | PCR\* | CRS\* | Total number of patient |
|----------------|--------|-------|-------|-------------------------|
| Positive       | Positive | Positive | Positive | 16                       |
| Negative       | Negative | Negative | Positive | 28                       |
| Positive       | Positive | Negative | Positive | 36                       |
| Negative       | Negative | Positive | Positive | 25                       |
| Negative       | Negative | Positive | Positive | 29                       |
| Negative       | Negative | Negative | Negative | 6                        |

*Total number of Xpert MTB/RIF positive cases - 36 (25.71%); \*Total number of MGIT-positive cases - 25 (17.85%); \#Total number of PCR-positive cases - 29 (20.71%); \#Total number of CRS positive cases - 118 (84.28%); PCR: Polymerase chain reaction, CRS: Composite reference standard

Table 3: Sensitivity and specificity of GeneXpert and polymerase chain reaction against gold standard as shown in table

| Test             | Sensitivity (%) | Specificity (%) |
|------------------|-----------------|-----------------|
| Xpert MTB/RIF    | 80 (86.89)      |                 |
| PCR              | 68 (89.57)      |                 |
| PCR: Polymerase chain reaction

Male:female ratio (1:1.5) of the patients in this study was quite consistent with the studies in Pakistan and India; however, differences were found with the studies done in UK and Pakistan.[22-25] Jha et al. found the number of patients having a cervical abscess or sinus quite low which was consistent with this study.[23] In our study, abscess was seen in 10% of cases and discharging sinus in 2.14% of cases while it was found in higher percentage in a study done in Bangladesh in which abscess was seen in 21.5% and sinus formation in 9.2% of...
Our study found unilateral neck swelling (87.14%) as the most common presentation which was similar to other studies. On chest radiograph, in our study, associated lung lesion was found in 12.86% of cases; whereas in other studies, it was seen in 16% and 7.5% of cases; while Choudhury et al. found associated lung lesions in 48.48% of cases. In our study, ESR was found raised in 47.85% of cases whereas Magsi et al. and Umer et al. found ESR raised in 12.5% and 47.7% of cases, respectively.

The diagnosis of EPTB is challenging for a number of reasons: the paucibacillary nature of the specimens, the lack of adequate sample amounts, or volumes, the apportioning of the sample for various diagnostic tests (histology/cytology, biochemical analysis, microbiology, and PCR), resulting in a nonuniform distribution of microorganisms.

Taking this into account, the present study was done to study the clinical profile and diagnostic modalities for diagnosing tuberculous lymphadenopathy. Among these 140 suspected cervical tubercular lymphadenitis patients, detection rates of MTBC by Xpert MTB/RIF, conventional PCR, and MGIT 960 were 25.71%, 20.71%, and 17.85%, respectively. Xpert MTB/RIF detected 16 cases which were negative by MGIT 960. There were five culture-positive cases, which were negative on Xpert MTB/RIF, this may be due to inhibition in the FNA sample. Although histopathology is an inexpensive and reliable tool for detecting tubercular lymphadenitis cases in resource-limited settings, studies have highlighted the limitations of associating specific histopathological features with TB. Nevertheless, histopathology can be invaluable in arriving at specific tissue diagnosis in diseases clinically mimicking TB such as lymphomas. Although the conventional methods (smear microscopy/culture) were used as a reference standard, these methods are not sufficient to detect all tubercular lymphadenitis cases as shown in our study. If we had gone by a single test, we would have missed true TB cases in which antituberculosis therapy was started on the clinical ground and improved.

Given treatment response in 11 cases, which were negative by all the microbiological investigations as well as inconclusive FNAC results, response to treatment adds to the clinical diagnosis. These limitations of all individual tests point toward the significance of CRS [Table 2]. Antituberculous chemotherapy is the mainstay in the management of TB lymphadenitis. The 6 months’ treatment may be sufficient for many patients (75%); however, it is difficult to define a clear-cut “end point” for assessing the efficacy of treatment of EPTB with delayed response. Six patients finally needed some form of surgical interventions to remove the lymph nodes. In a study by Jha et al., most of the patients treated successfully with short-course chemotherapy for 6 months while surgery was required sometimes.

**Conclusion**

Clinicians often face the diagnostic dilemmas presented in the study. Individual modalities of diagnosis are available but all have drawbacks with varied sensitivity and specificity. Combining the available clinical, radiological, and microbiological modality to reach early diagnosis can go a long way to avoid misdiagnosis and unnecessary delay in treatment, especially in cases, without pulmonary involvement and fulfilling the aim of national tuberculosis control program for EPTB cases. In the current study, CRS criteria were better than conventional method/s for the diagnosis of cervical lymphadenitis cases, and early initiation of antitubercular therapy reduces the transmission of TB in community due to high chances of concurrent pulmonary tuberculosis.

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**Conflicts of interest**

There are no conflicts of interest.

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