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Spinal Tuberculosis : Modern Laboratory Based Approach, An Experience From Tertiary Care Hospital From India

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ABSTRACT

A total of 50 patients diagnosed with spinal tuberculosis at Seven Hills Health Care City, Mumbai, India during 2011 - 2013 were retrospectively analysed to investigate culture positivity rate and utility of Line Probe Assay (LPA). Microscopy was positive only in 14(28%) and culture was positive in 35(70%) cases. Sensitivity testing was done by Line probe assay in 18 cases and phenotype method in 3 cases. MDR TB was detected in 2(9.5%) and INH monoresistance in 3(14.28%) cases. The Lineprobe assay report was available to clinician within 48 hrs and anti-TB therapy was tailored accordingly. These 21 patients recovered clinically and radiologically except 1 patient who was lost on follow up. Considering the available methods, the ideal protocol for spinal tuberculosis would be to detect MTB complex from sample, culture, Line probe assay for MDR/XDR detection from culture positives to initiate appropriate therapy followed by phenotype sensitivity testing.

KEYWORDS : Spinal tuberculosis, genotype sensitivity, MDR TB detection

Introduction : Spinal tuberculosis is a frequently encountered extrapulmonary form of the disease. The disease is popularly known as Pott's spine. The exact incidence and prevalence of spinal tuberculosis in most parts of the world are not known. In countries with a high burden of pulmonary tuberculosis, the incidence is expected to be proportionately high. Approximately 10% of patients with extrapulmonary tuberculosis have skeletal involvement. The spine is the most common skeletal site affected, followed by the hip and knee. Spinal tuberculosis accounts for almost 50% cases of skeletal tuberculosis (Cengizhan Sezgi et al, 2014). The diagnosis of extra-pulmonary tuberculosis (EPTB) is a major challenge due to the difficulty in obtaining appropriate clinical sample, especially from the nonreachable sites and poor sensitivity of diagnostic tests (Manuel Fuentes Ferrer et al, 2012). This study aimed to investigate culture positivity rate and utility of Line Probe Assay (LPA) among spinal tuberculosis cases.

Methods : Patients diagnosed with spinal tuberculosis at Seven Hills Health Care City, Mumbai, India during 2011 - 2013 were enrolled in the study retrospectively. Spinal TB was diagnosed if at least one of the following criteria was met:

- Mycobacterium tuberculosis or acid-fast bacilli (AFB) in spinal tissue, paraspinal tissue, or a psoas abscess.
- Histopathological evidence of TB in spinal tissue including caseating granulomas with or without a positive AFB smear.
- Highly probable diagnosis of spinal TB supported by radiological findings via X-ray, CT, and MRI or clinical features correlating with spinal TB.
- The confirmed diagnosis was established when *M.tuberculosis* was isolated from pus, tissue, bone biopsy specimen.

Components of each patient's clinical workup were also investigated and consisted of laboratory results, imaging results, histopathological findings, and microbiological results.

The cultures were performed using BACT/ALERT 3 D (Biomeieureux, France) automated system as per the standard protocol. Smears were stained using Zeil Neilson method and Auramine staining (Flourescent staining). The prelimnary smear report was communicated if positive. The confirmation of Mycobacterium tuberculosis complex was done using MPT 64 antigen (Standard Diagnostics) test. The confirmed results were communiacated to clinicancian requesting Line probe assay (HAIN diagnostics, Germany) and phenotype sensitivity testing. The Line probe assay results were available to the clinican within 48hrs.

Results and discussion: A total of 50 patients including 28 (56%) men and 22 (44%) women aged between 18 to 81 years (mean 41.7). Back pain was the commonest symptom. One patient had quadriparesis. Commonest site of vertebral involvement was observed at lumbar region (n=23, 46%) and thoracic (n=19, 38%) respectively (table 1). The distribution of sample type and laboratory finding is shown in table (2). Concomittant pulmonary infection was noted in 3 cases. Microscopy was positive only in 14 (28%) cases and culture was positive in 35 cases (70%, n=50). Of the 15 culture negative cases, 3 were on anti-TB therapy and another 6 cases did not have findings suggestive of TB on histopathological examination as well. Sensitivity testing was done by Line probe assay (MTBDR plus assay) in 18 cases and phenotype method in 3 cases. MDR TB was detected in 2 case (9.5%, n=21) and INH monoresistance in 3 cases (14.28%). All 35 patients were treated with empirical anti-TB therapy. Two required abscess drainaige and 3 required additional surgical intervention.

Spinal tuberculosis is due to haematogenous dissemination from a primary focus. The infection reaches the skeletal system through vascular channels, generally the arteries, as a result of bacteremia, or rarely in the axial skeleton through Batson's plexus of veins. The infection spreads to the adjacent vertebral bodies under the longitudinal ligaments. The most common site is the thoracolumbar junction, but any segment of the spine can be involved. Atlantoaxial junctions are the least common sites. The symptoms are back or neck pain and non specific complaints such as weight loss and fever; thus, the diagnosis can reliably be made on a clinical and radiological basis, if the disease is suspected and , microbiology access can increase diagnostic accuracy. The infection begins in the cancellous area of the vertebral body, commonly in the epiphyseal location and less commonly in the central or anterior area of vertebral body. The infection spreads and destroys the epiphyseal cortex, the intervertebral disc and the adjacent vertebrae. The vertebral body becomes soft and gets compressed to produce either wedging or total collapse. Anterior wedging is commonly seen in the thoracic spine where the normal kyphotic curve accentuates the pressure on the anterior part of vertebrae. The exudate penetrates the ligaments and follows the path of least resistance along fascial planes, blood vessels and nerves, to distant sites from the original bony lesion as a cold abscess. Paraplegia (Pott's paraplegia) is the most serious complication of spinal TB and its occurrence is reported to be as high as 30 percent in patients with spinal TB (Fraser Wares et al)

In our study there is not a big difference between the proportion of spinal TB in men and women; however, some studies have reported a male predominance . Spinal TB occurs mostly during the first three decades of life. In developed countries the disease is reported more frequently in the elderly. MRI and CT are actually the most useful methods for detection of spinal lesions, especially in early stages of the disease MRI has proved to yield a higher diagnostic accuracy than

CT scan . Radiologically guided needle aspiration (RGNA) is a simple, reliable, and practical approach for diagnosing TS, since it provides useful material for histopathological and microbiological studies. It is particularly indicated for patients who are candidates to chemotherapy alone, and it may also be used for drainage of paraspinal abscesses. Open surgical biopsy may be necessary if needle biopsy is unsuccessful, or in the cases in which surgical intervention is indicated for treatment.

The diagnosis of extra-pulmonary tuberculosis (EPTB) remains an important clinical problem, due to low bacterial load in extra-pulmonary specimens. The main limitation of the culture test is its slowness in revealing positive results determined by the metabolic characteristics of the pathogen and the number of bacteria present in the sample (G. Delgou et al, 2012). In our study culture positivity rate was 70% (n=35) and isolation rate was good from pus and tissue samples compared to bone biopsy samples. A recent report by Kumar et al, where 51 patients with Pott's disease were evaluated, indicated that the sensitivity of the culture 43% (Parveen Kumar et al, 2014). In another Indian study the culture sensitivity rate was 53% for extrapulmonary specimens (Viral Vadwai et al, 2011) and many of the patients enrolled in that study were on antituberculous therapy.

The nucleic acid amplification techniques are rapid and sensitive has modified strategies for the detection of *Mycobacterium tuberculosis.* Since the application of the polymerase chain reaction (PCR) in the diagnosis of TB, diagnostic protocols using varying methods of DNA purification and different *M. tuberculosis* target sequences have been evaluated for extra pulmonary tuberculosis (WHO, 2013). The recently developed CE-marked Xpert MTB/RIF (Xpert) test (Cepheid Inc.), based on nested real-time PCR and molecular beacon technology, is a point of care rapid test, with a result for TB and RIF resistance within 2 hours and has a high sensitivity in smear-negative pulmonary TB (Doris Hillemann et al, 2011) . A series of meta-analyses has shown that nucleic acid amplification tests (NAATs) have high specificity and positive predictive value with highly variable sensitivity, especially in cases of EPTB Ahmad (Naeem Sajed et al, 2014). Indian study by Vadwani etal shows that the Xpert test has true diagnostic potential with good sensitivity 96 % for pus; and moderate sensitivity (77%) for tissues. These findings are similar to study done by Doris Hillemann et al which showed 69% sensitivity with tissue specimens. Held M et al showed test showed a sensitivity of 95.6% and specificity of 96.2% for spinal TB by GeneXpert test and results were available within 48 hours compared with a median of 35 days for cultures. Though the molecular detection directly from the sample was not done in our study, the recent findings are really encouraging to use this as one of the important tool to get the confirmed results within 2 hours pending culture reports. Histopathology report takes atleast 3-4 working days. However, Histology is important in differential diagnosis. Though culture takes a long time atleast 4-5 weeks for EPTB samples it is essential for sensitivity testing and gold standard for EPTB diagnosis.

The phenotypic sensitivity from culture positives takes atleast minimum of 2-3 weeks and the Line probe assay (MTBDR plus assay) takes just 48hrs for MDR/XDR detection. LPAs use multiplex polymerase chain reaction (PCR) amplification and reverse hybridization to identify M. tuberculosis complex and mutations to genes associated with rifampicin and isoniazid resistance. LPA can be performed directly from acid fast bacilli (AFB) smear-positive sputum, or from culture isolates, and provide results in 1-2 days. It is highly sensitive and specific for detection of rifampicin resistance (≥97% and ≥99%) and isoniazid resistance (≥90% and ≥99%) on culture isolates and smear-positive sputum. Overall agreement with conventional DST for detection of MDR-TB was 99% (Parveen Kumar et al, 2014) . On GeneXpert , MDR is predicted if Rifampicin is resistant. Mono resistance to Rifampicin and INH is known (Ali Akbar Velayati et al, 2014). Rifampicin monoresistance was not noted in our study. Sensitivity testing was possible only in 21(60%) cases. MDR TB was detected in 2 case (9.5%, n=21) and INH monoresistance in 3 cases (14.28%) by LPA and the results were available within 48 hours to the clinician and anti-TB therapy was tailored accordingly. The MDR TB detected cases did not have past history of antituberculous treatment. Management of spinal tuberculosis requires treatment with standard four drug anti-tuberculosis treatment (ATT) regimen in the drug susceptible cases (isoniazid, rifampicin, ethambutol and pyrazinamide in the intensive phase followed by isoniazid and rifampicin in the continuation phase). In case of drug resistant tuberculosis the regimen has to be adjusted appropriately. The optimal duration of ATT in spinal tuberculosis treatment is controversial. The current World Health Organisation (WHO) guidelines recommend 9 months rifampicin based regimens for the treatment of spinal and other osteoarticular tuberculosis because of the difficulties in assessing the treatment response. In spite of these recommendations, longer duration of therapy (12-18 months) is still regarded as a standard of care by many orthopaedic surgeons in endemic areas. Bracing of spinal tuberculosis patients helps in arresting the curve progression, provides pain relief and encourages early ambulation. Surgery may be indicated in treatment of spinal tuberculosis to relieve the neurological symptoms, prevent the progression and correction of deformities. These 21 patients recovered clinically and radiologically except 1 patient who was lost on follow up.

Considering the available methods, the ideal protocol for spinal tuberculosis would be to detect MTB complex from sample, culture, Line probe assay for MDR/XDR detection from culture positives to initiate appropriate therapy followed by phenotype sensitivity testing.

Table 1: Vetebral involvement

Lumbar	23 (46%)
Thoracic	19(38%)
Multiple level	5 (10%)
Sacral	2 (4%)
Cervical	1 (2%)

Table2 : Laboratory findings

Sample types (n=50)	Microscopy results (n=50)		Culture results (n=50)	
	Positive	Negative	Positive	Negative
Pus (n=21)	8	13	18	3
Tissue (n=18)	6	12	11	7
Bone biopsy (n=11)	0	11	6	5
	14 (28%)	36	35 (70%)	15

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