Musculoskeletal tuberculosis - A diagnostic dilemma: Clinico-bacteriological study among patients attending tertiary heath care centre in North Eastern India

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Abstract

Introduction and Objectives: Clinicians consider musculoskeletal tuberculosis as diagnostic dilemma because diagnosis frequently gets delayed due to its varied clinical presentation, false negative results on smear microscopy and less sensitivity on culture methods (20-40%). The main objectives were to know the prevalence of musculoskeletal tuberculosis (TB) among patients attending NEIGRIHMS and to understand clinical bacteriological profile of musculoskeletal tuberculosis using conventional and molecular laboratory diagnostic methods.

Materials and Methods: In this study, a total of fifty two clinical specimens like pus swab, ultrasonography guided (USG) pus aspiration, synovial fluid and bone biopsy of newly suspected musculoskeletal tuberculosis patients were evaluated for microscopy, culture and molecular detection of *M. tuberculosis.*

Results: The study reveals prevalence of 46.2% musculoskeletal tuberculosis among the clinically suspected patients. The most commonly involved sites in suspected musculoskeletal tuberculosis were hip joint (23.1%). USG guided pus comprises only 26.9% of the samples collected, however, yield of *M. tuberculosis* from them were 71%. PCR detected the maximum number of cases of musculoskeletal tuberculosis 24 (46.2%) followed by culture method 13 (25%) and smear microscopy 1 (1.92%).

Conclusion: This observation will help guiding clinicians in effective management of musculoskeletal tuberculosis cases because delayed diagnosis and treatment in musculoskeletal TB results in poor outcome.

Keywords: Extrapulmonary tuberculosis, Osteoarticular, Polymerase Chain reaction.

Introduction

In Extrapulmonary TB (EPTB) highly vascular areas such as lymph nodes, pleura, genitourinary tract, bones and joints and meninges are commonly affected.[1] Although musculoskeletal TB comprises 10-15% of all extrapulmonary TB, it is difficult to diagnose with conventional bacteriological methods like acid fast staining, fluorescent microscopy and culture methods due to lack of representative samples.[2] PCR is highly sensitive, specific and rapid diagnostic modality for *Mycobacterium tuberculosis.*[3] The diagnostic dilemma and the challenges are many: firstly, skeletal TB is a paucibacillary condition; secondly it mimics other common musculoskeletal conditions like bone tumors, rheumatoid arthritis, osteoporotic vertebral fracture, and other infective conditions.[4] Finally, paucity of proper microbiological settings in the north eastern region and lack of specialized persons for collection and handling of laboratory samples needs urgent attention as there is paucity of studies to the best of our knowledge on musculoskeletal tuberculosis. For instance, multi-drug resistant (MDR) tuberculosis cases need drug sensitivity testing based on quality culture methods in an accredited laboratory that are currently nonexistent in many states of the north east India. Considering the fact mentioned above the present study has been undertaken with the intent to study the prevalence of musculoskeletal tuberculosis among the patients attending tertiary health care centre and to provide a rapid and reproducible methodology for understanding clinic-bacteriological profile and making definitive diagnosis of musculoskeletal tuberculosis using microscopy, culture and PCR assays of pus, synovial fluid or other tissue samples, obtained in a less invasive manner whenever feasible, from clinically suspected musculoskeletal tuberculosis patients.

Materials and Methods

A total of fifty-two clinically suspected musculoskeletal tuberculosis patients fulfilling the inclusion criteria based on signs and symptoms such as pain, restricted joint movements, swelling of the joints and other constitutional symptoms were included in this study. The study was conducted after obtaining informed consent of the participant on approval of the ethical clearance of the institute. The collected specimens like pus swab, USG guided pus aspirate, synovial fluid and bone biopsy materials were processed and subjected for smear microscopy for demonstration of Acid Fast Bacilli (AFB), isolation of *M. tuberculosis* through in-vitro culture and molecular analysis employing PCR assay for confirmation of laboratory diagnosis using laboratory diagnostic techniques described elsewhere. The decontaminated and concentrated sediment was used for microscopic examination of AFB, culture on Lowenstein Jensen (LJ) slope, and PCR.[1,3,4] *M. tuberculosis* H37RV strain was used as control strain obtained from Intermediate

Reference Laboratory (IRL), Guwahati under RNTCP (Revised National Tuberculosis Control Programme), Government of India.

PCR for TB was done using a specific sequence *IS6110* AmpliSensÒ *MBT*-EPh PCR kit (REF B15-100-R0, 5-CE, AMPLISENS, Moscow, Russia) having two oligonucleotide primer IS6110f (5'- GGC AAA GCA GCT CTC TCT GC-3') and IS6110r (5'- GGA CTG CCA CCT TCC ATC TTC-3') in thermocycler DNA PCR machine (CG Palm Cycler 9600, Genetix Biotech, New Delhi) which were incorporated in PCR tubes.. PCR was carried out employing utmost precautions to prevent cross- and carryover contamination. Genomic DNA was extracted from various specimens using DNA-sorb-B nucleic acid extraction kit (REF K1-2-100-CE, AMPLISENS, Moscow, Russia) as per manufacturer's protocol. *Mycobacterium tuberculosis* DNA was amplified from 10µl of extracted sample using AmpliSens*MBT*-EPh PCR kit as per Manufacturer's Protocol. Amplified DNA was electrophoresed using 1.7% agarose gel with ethidium bromide at 90 Volts for 1 hr and the resultant bands were interpreted by UV transillumination.

Results

The study shows the prevalence of 46.2% musculoskeletal tuberculosis among the clinically suspected patients of musculoskeletal tuberculosis and indicated slight preponderance of females (54.2%) in musculoskeletal tuberculosis than males (45.8%). Majority of the patients affected by musculoskeletal tuberculosis were in the age group of 21-30 years (28.9%) followed by pediatric age group (17.3%).

The most commonly involved sites in suspected musculoskeletal tuberculosis were hip joint (23.1%) and knee joint (23.1%) followed by lumbar spine (19.2%) and long bones of lower limb (17.4%). Majority cases of musculoskeletal tuberculosis involved Hip joint 10 cases (41.67%), with equal involvement of left and right side. Knee joint was involved in 25% and lumbar spine in 12.5% cases of musculoskeletal tuberculosis. Long bones of lower limb like tibia and femur were involved in 16.6% cases of musculoskeletal tuberculosis.

Among 52 clinical samples of suspected musculoskeletal tuberculosis patients, pus swabs 23 (44.2%) were collected from joints like hip joint, knee joint, ankle joint and discharge from bones like tibia, femur, spine, upper limb bones etc, followed by ultrasonography guided pus aspiration (26.9%), synovial fluid (21.2%) and bone biopsy materials (7.7%). USG guided pus comprise only 26.9% of the sample collected however, yield of *M. tuberculosis* from them were 71%, making them better samples in comparison to pus swab which had a poor yield of 30.4%. Samples like synovial fluid and bone biopsy showed significant detection of *M. tuberculosis* with positive percentage of 45.5% and 50% respectively.

All 52 clinical specimens from newly suspected musculoskeletal tuberculosis were subjected for conventional and fluorescent microscopy (Image 1 and 2), however only 1 USG guided pus aspirate from hip joint demonstrated AFB (1.92%). There was no significant difference between the detection of tubercle bacilli by conventional and fluorescent microscopy.

Among all samples subjected for in-vitro culture on LJ slant, 13 clinical specimens (25%) demonstrated growth of colonies. USG guided pus aspirate and bone biopsy materials from hip joint (46.2%) reported maximum growth on LJ slant. Samples collected from knee joint reported 33.3% growth on culture media.

In the present study, PCR detected the maximum number of cases of musculoskeletal tuberculosis 24 (46.2%) followed by culture method 13 (25%) and smear microscopy 1 (1.92%). Among the 39 suspected musculoskeletal tuberculosis cases which were negative on conventional methods, 11 cases (28.2%) were found to be positive on PCR(Image 3).

Discussion

Osteoarticular tuberculosis can cause significant morbidity and a high index of suspicion is needed for early diagnosis so as to avoid bone destruction and disability.[2] In the study, female preponderance (54.2%) was seen in musculoskeletal tuberculosis compared to males (45.8%). Such observation did not reveal any significant difference of disease in male or female. Our observations were in concordance with that of Yoon et al[5] and Jutte et al.[6] However, some other studies by Nasiri et al[7] and Enache et al,[8] reported a complete opposite finding that preponderance among males was higher compared to females.

Among 52 cases of clinically suspected musculoskeletal tuberculosis, the most commonly affected age group was between 21- 30 years (42%) followed by pediatric age group (20.2%). This observation was similar to the findings of Arathi et al[9] (37%) and Sharma et al[10] (40%). However, there was difference in the observation with the study of Enache et al[8] (21%) where the mean age group was more than 50years.

In our study highest numbers of the cases were represented by suspected patient with joint tuberculosis - 29 cases (55.8%) where hip joint - 12 cases (23%), knee joint - 12 cases (23%) and ankle joint-5 cases (9.6%). The study indicated highest detection of 10 cases (41.67%) of tubercular lesions affecting the hip joints with equal involvement of both right and left side. The involvement of hip joint in present study was in accordance with the study reported by Enache et al[8] (31%) and Sharma et al[10] (27%). The findings for Hip joint tuberculosis were inconsistent with the studies of Ruiz *et al*[11] (15.4%) and Sandher et al[12] (2.38%). Our study reported 6 cases (25%) of knee joint tuberculosis with equal predilection for right and left side, among the 24 positive musculoskeletal cases. This observation

was in accordance with the study of Sharma et al[10] (10.88%) and Prakash et al[13] (19%). However these findings differ from the study in Madrid by Arathi et al[9] (43.75%) and Ruiz et al**[11]** (57%) were high percentage of knee joint involvement was reported. In our study 10 cases (19.2%) were suspected of lumbar spine tuberculosis among 52 cases of musculoskeletal tuberculosis. The study reported 3 cases (12.5%) with tubercular lesion affecting lumbar spine, this observation was similar to the study of Ruiz et al[11] (15.4%). However, these observations vary from the study of Sharma et al[10] (50.77%), Sandher et al**[12]** (44%). The reason for this variation could be difference in geographic and genetic variability of the study population.

In our study we found that 45 patients (90.4%) presented with pain and difficulty while walking, followed by backache in 29 patients (55.8%) and discharge from joints in 25 patients (48.1%). These findings compare favorably with those of Arathi et al[9] and Ruiz et al[11] where pain (83%), was the presenting complaint in majority of the patients. Constitutional symptoms of tuberculosis like fever and weight loss were reported in 24 (46.2%) and 22 patients (42.3%) respectively which was consistent with the study of Sharma et al[10] (45%), Sandher et al[12] (39%).

In this study, 52 new patients with clinical suspicion of musculoskeletal tuberculosis were evaluated by conventional and fluorescent microscopy for the presence of acid fast bacilli by Ziehl-Neelsen and auramine staining methods. Out of 52 samples received, only 1 USG guided pus aspirate sample yielded Acid Fast bacilli (1.92%) and 51 (98.1%) were negative in smear microscopy. These findings were similar with those of Prakash et al[13] (0%) and Ganavalli et al[14] (1.64%). However, these observations differ from the study conducted in Moradabad, India by Arathi et al[9] (25%) and in Greece by Verettas et al**[15]** (33.3%). The reason for such difference in the findings was small sample size in the later studies. The standard guideline says that high bacterial load (10^4 – 10^5 bacilli/ml) is needed in the specimen to render an AFB microscopy result positive.

Conventional culture on Lowenstein-Jensen medium using NALC-NaOH decontamination method yielded pure growth in thirteen (25%) specimens. Maximum growth on LJ slant was seen in fourth week of aerobic incubation (69.2%) followed by 2 specimens (15.4%) in third week of aerobic incubation. Among 13 positive culture specimens, Twelve specimens were smear negative and one smear positive, the negative smear result being explained by very low bacillary counts in the samples. The findings of our study was in agreement with the results reported by Apurba SS et al[16] (21.4%) and Wang G et al[17] (26.92%). However, contrary to our findings, study conducted in Karnataka, India by Ganavalli et al[14] reported lower detection of *Mycobacterium tuberculosis* by LJ culture 6.25%. This fact indicates that culture was indeed more sensitive than smear microscopy and rightly considered as the gold standard. One smear positive sample showed growth on LJ culture slant after 4weeks of aerobic incubation.

In this study we have attempted to investigate the relevance of PCR assay in clinically suspected cases of musculoskeletal tuberculosis. In the present study (Table 1) the high degree of detection was apparent in the actual clinical setting, since PCR detected 24 (46.2%) among 52 cases studied, of which culture was positive in only 13 cases (25%) and smear microscopy detected AFB in one case (1.92%). The findings of PCR in this study compare favourably with Prakash J et al[13] (40.1%) and Muangchan et al[18] (33.3%). However, differs from the study of Pandey V et al[19] (70.83%) and Negi SS et al[20] (78.2%). Among the 23 pus swab samples, only 3 (13%) samples showed growth of *M. tuberculosis*, smear microscopy did not detected any AFB from pus swab, however, PCR assay reported 7 pus swab samples (30.4%) positive for *M.tuberculosis.* Among the 20 pus swab specimens which were negative by conventional staining and culture methods, PCR detected 4 positive cases (20%) of musculoskeletal tuberculosis. The findings related to poor yield of pus was also reported in the study conducted by Ganavalli et al[14] (25.8%) and Maurya et al[21] (27.65%), However, these findings were inconsistent from the study of Ruiz et al[11] (66.67%) and Prakash et al[13] (43.24%). In the study 14 USG guided pus aspirate samples were collected from suspected musculoskeletal tuberculosis patients, among them 1 (7%) was positive on smear microscopy and 6 (42.9%) were positive on LJ culture medium, however, PCR demonstrated 10 positive (71.4%) samples. Among the 8 USG guided pus aspirate samples which were not demonstrated on conventional and fluorescent microscopy and culture, PCR reported 4 positive samples (50%). Among the 12 cases of suspected hip joint tuberculosis, only 1 case (8.33%) demonstrated AFB on smear microscopy and 6 cases (50%) showed growth on LJ culture slant. However, PCR detected 10 cases (83.33%) positive for *M.tuberculosis* among the 12 suspected cases of hip joint tuberculosis. In 6 suspected hip joint tuberculosis cases, which were negative by conventional methods, 4 (66.7%) were detected positive by PCR for Hip joint tuberculosis. In our study, among 12 suspected knee joint tuberculosis cases 4 (33.3%) were positive on culture, however 6 (50%) were detected by PCR. In the present study 10 cases of lumbar spine suspected with vertebral tuberculosis were studied. Among 10 cases, 1 case (10%) demonstrated growth on culture, however, 3 cases (30%) were positive by PCR method. In suspected lumbar spine tuberculosis 9 cases were negative on conventional microscopy and culture methods, however, 2 cases (22.2%) were detected positive on PCR assay. This observation was similar to

the study of Arathi et al[9] (18.75%) and Ruiz et al[11] (15.4%). However, these observations vary from the study of Sharma et al[10] (50.77%) and Sandher et al[12] (44%).

The PCR was positive in 11(28.2%) specimens which were negative by both conventional bacteriological techniques (Table 2). This finding was consistent with the study of Kumar et al[22] (26%). Analysis of PCR results among specimens that were positive and negative by conventional bacteriological methods showed that one pus specimen was positive on smear microscopy and this specimen was positive on PCR (100%). Among the 51 negative specimens by smear microscopy, 23 (45.1%) of these specimens were positive on PCR. This observation was in corroboration with the studies of Kumar et al[22] (96% in smear positive specimens and 46.3% in smear negative specimens) and Negi SS et al[20] (100% in smear positive specimens and 35% in smear negative specimens). Similarly, in the present study PCR had 28.2% positivity among culture negative specimens and 100% positivity in positive specimens. These findings compare favorably with Negi SS et al[20] and Kumar et al.[22]

Fig. 1: Conventional Microscopy showing scattered Acid Fast Bacilli on Ziehl Neelsen Staining

Fig. 2: Lowenstein Jensen culture medium showing growth of *Mycobacterium tuberculosis* colonies from

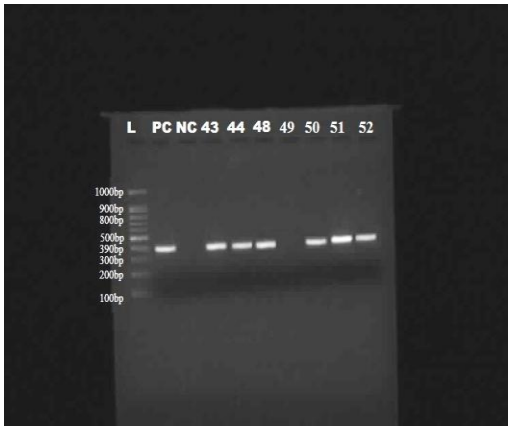

Fig. 3: PCR amplification of 390bp in IS6110 region of *M.tuberculosis* on 1.7% agarose gel [L: DNA ladder 1000bp to 100bp; PC: Positive Control; NC: Negative control] Lane 49: Negative, Lane 43, 44, 48, 50, 51, 52: Positive

Table 1: Comparative results of Staining, Culture and PCR in Study population

Technique (n=52)	Positive	Percentage	Negative	Percentage
Smear Microscopy	1	1.92%	51	98.07%
Culture on LJ Media	13	25%	39	75%
Polymerase Chain reaction	24	46.2%	28	53.8%

Table 2: Comparison of PCR results with other tests performed in the patients with suspected musculoskeletal tuberculosis

Test result other than PCR (No. of Samples)	PCR Result	
	Postive=n (%)	Negative=n (%)
Smear positive (1)	1(100%)	0
Smear negative (51)	23(45.1%)	28(54.9%)
LJ Culture Positive (13)	13(100%)	0
LJ Culture Negative (39)	11(28.2%)	28(71.8%)
Smear positive, culture positive (1)	1(100%)	0
Smear negative, culture positive (12)	12(100%)	0
Smear negative, culture negative (39)	11(28.2%)	28(71.8%)

Conclusion

The findings in our study can act as a bridge between early diagnosis of musculoskeletal tuberculosis at community or primary health care centre and prevention and treatment of deformity at the earliest possible, as these health care centres lack facility for clinical specimen collection from deep joints and diagnosis by conventional methods is difficult. Although the sample size in our study was small, it still highlights the importance of taking up more studies on a larger scale in this spectrum to help correlate the facts, uncover newer epidemiological and clinically relevant knowledge and thereby define the exact impact of the disease in our society and novel ways to decimate the same.

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Conflicts of interest

There are no conflicts of interest.

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