Original Article

Use of amplified *Mycobacterium tuberculosis* direct test (Gen-probe Inc., San Diego, CA, USA) in the diagnosis of tubercular synovitis and early arthritis of knee joint

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Abstract

Background: The diagnosis of knee joint tuberculosis, especially in early stages of synovial disease, has more often been based on clinicoradiological suspicion, with no single test claiming to be a dependable rapid diagnostic test with high sensitivity and specificity. Nuclear amplification tests in vogue like the polymerase chain reaction have shown variable sensitivity and false positivity rates in various studies. We evaluated the role of Amplified *Mycobacterium tuberculosis* Direct Test (AMTDT) or Genprobe in the diagnosis of knee joint tuberculosis in early, especially, early synovitis and arthritis cases.

Patients and Methods: Thirty two patients of suspected knee joint tuberculosis were subjected to diagnostic arthroscopy during the study period. The synovial fluid and tissue were subjected to mycobacterial culture, histopathology, and AMTDT. A comparative analysis of the sensitivity and specificity of this new test with culture and histopathology was done and the time taken for reporting was calculated for each test.

Results: Out of 32 tissue samples, 8 were found to be positive with mycobacterial culture [Lowenstein Jensen (LJ)/Bactec], 11 were positive with histopathology, and 5 were found to positive with AMTDT. The sensitivity of AMTDT was found to be 62.5% and specificity was 100% with a *P* value of 0.083. The results were obtained earliest with AMTDT with a mean reporting time of 1.2 days, while the results of histopathology were obtained in a mean time of 6.8 days, BacT alert in 22.5 days, and conventional LJ medium culture results in 48.6 days.

Conclusion: AMTDT or Genprobe is a rapid diagnostic test for early diagnosis of tubercular arthritis, but has low sensitivity in knee joint tuberculosis. Nuclear amplification tests are still far from being a single promising alternative to conventional tests in cases of joint tuberculosis. Routine use of arthroscopic biopsies in all suspected cases is helpful in the early diagnosis of knee joint tuberculosis.

Key words: AMTDT, arthroscopy, Genprobe, tubercular arthritis

Introduction

Osteoarticular tuberculosis (TB) constitutes about 10% of extrapulmonary TB, which is about 1–3% of total TB cases.[1] Tubercular synovitis and early arthritis have been called as "the great imitator" due to atypical clinical presentation and lack of specificity in diagnosis.[2,3] Early diagnosis and timely institution of antitubercular treatment (ATT) is crucial as delay leads to irreparable damage to the joint and restriction of joint mobility. The diagnosis of joint TB in endemic areas has more often been based on clinicoradiological suspicion with empirical trial of ATT.[4,5] It is justified to treat the patients empirically only in cases with classical clinical and X-ray appearance of tubercular lesions of the bone, which is usually not the case, especially in synovial disease and early arthritis.

Conventional microbiological methods like smear and culture have low sensitivity and specificity, especially in synovial TB due to the paucibacillary nature of disease.[6,7] In addition, the culture of *Mycobacterium tuberculosis* is time consuming, taking 6–8 weeks for the growth to appear and much longer time for positive growth, especially in paucibacillary cases like joint TB.[8-10] Nucleic acid amplification (NAA) methods have the potential to reduce detection and identification time to within hours of sample

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collection.[10,11] The Amplified *Mycobacterium tuberculosis* Direct (AMTDT) test (Gen-probe Inc., San Diego, CA, USA) approved by FDA in 1998 is an isothermal transcription-mediated amplification system based on the reverse transcription of mycobacterium-specific rRNA targets, followed by transcription of the DNA intermediate template, which are subsequently detected by *M. tuberculosis* complex specific DNA probe. AMTDT has shown promising results in a variety of extrapulmonary samples like cerebrospinal fluid (CSF), lymph node, and gastric washings by other researchers,[10-14] but no study as yet has evaluated the efficacy of this test in synovial TB. Hence, this study was carried out to evaluate the usefulness of AMTDT in all suspected cases of early tubercular arthritis by subjecting them to arthroscopically guided synovial biopsies.

Patients and Methods

Ethical approval was obtained from the institutional board and written informed consent was taken from all patients.

This was a prospective study that was carried out between September 2007 and December 2009. A total of 32 patients of clinically suspected tubercular synovitis and early arthritis of knee joint were included in the study. The detailed clinical presentations of the patients, including demographic data, duration of symptoms before seeking medical attention, presenting symptoms, and relevant history, were recorded and a detailed physical examination was performed.

Anteroposterior and lateral radiographs of the affected joint were obtained and classified according to the staging system of Kerri and Martini.[15] Routine blood investigations including hemogram, erythrocyte sedimentation rate (ESR; Wintrobe method), and qualitative C-reactive protein (CRP) and liver function tests were performed for all patients. All patients had a chest radiograph, histologic examination of sputum for acid-fast bacilli (AFB), and enzyme-linked immunosorbent assay for human immunodeficiency virus (HIV).

All patients with more than one joint involvement, small joints involvement, history of ATT taken in the recent past, advanced radiological changes (Kerri and Martini stage 3 and 4), and HIV seropositivity were excluded from the study.

Diagnostic arthroscopy was performed under anesthesia using a 4-mm oblique forward (30°) telescope. Synovial biopsy was taken from the inflamed areas [Figure 1] by double puncture technique using punch biopsy forceps. Samples for histopathology were fixed in 10% formalin, while samples for bacteriological studies were diluted in normal saline. All the samples were subjected to AMTDT, histopathologic evaluation, and microbiological smear and culture by Lowenstein Jensen (LJ) medium as well as colorimetric BacT alert 3D system.

The specimen preparation and performance of test was done in accordance with the manufacturer's manual.[16]

Sample preparation and detection

The specimen decontamination was done as per the standard Centers for Disease Control and Prevention (CDC) protocol using 1–1.5% NaOH for 15–20 min, and then centrifuged at 6000 rpm for 20 min.

The decontaminated samples and controls were placed into the mycobacterium lysing tubes and 50 μL of mycobacterium Specimen Dilution Buffer (SDB) was pipetted into each of the tubes. The tubes were then sonicated for about 15 min, thus converting the sample and controls into "lysates." The stringency of test controls was maintained within the milieu of the equipment as it was a closed system. The amplification tubes were then prepared for each of the test sample, positive and negative control lysates. Lyophilized *Mycobacterium tuberculosis* Hybridization Reagent (H) was added to each tube using a repeat pipette. The tubes were then incubated at 60°C for 15 min and 300 μL of mycobacterium Selection Reagent (S) was added to each of the tubes. The tubes were then incubated at 60°C for 15 min and placed in the Genprobe LEADER Luminometer and the reading was taken in relative light units (RLU). The procedural time for AMTDT was about 3–5 h for a single sample. A reading of ≥500,000 RLU was taken as positive for *M. tuberculosis* complex rRNA, while <30,000 RLU was taken as negative as recommended by Middleton *et al*.[12]

Histopathologic evaluation

All the formalin-fixed tissues received for histopathology were embedded in paraffin, cut to 5-μm-thick sections, and were stained with hematoxylin–eosin (H&E) before microscopic examination. The stained slides were examined carefully for any evidence of granulomatous inflammation with or without casseous necrosis [Figure 2].

Microbiological culture and BacT alert

Approximately 3 mL of synovial tissue was centrifuged at 3000 rpm for 20 min and the deposit was inoculated into LJ medium and MB/BacT culture bottles. Growth in MB/BacT bottle was detected through the carbon dioxide that was released during the metabolic process detected by a colorimetric sensor.

Results

Diagnostic arthroscopy and arthroscopic synovial biopsy was

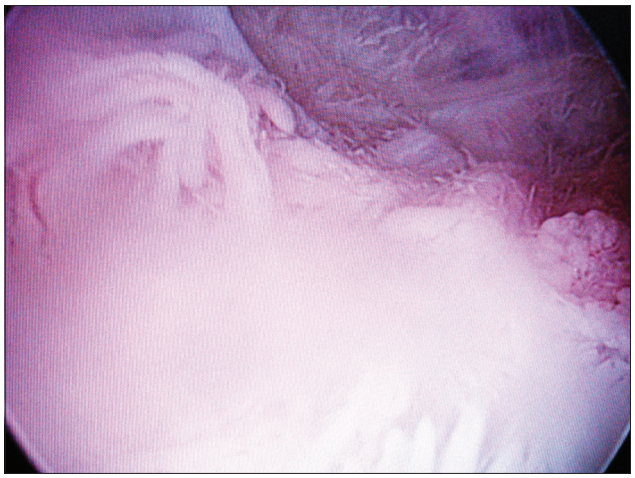

Figure 1: Arthroscopic view of inflamed synovium in tubercular synovitis

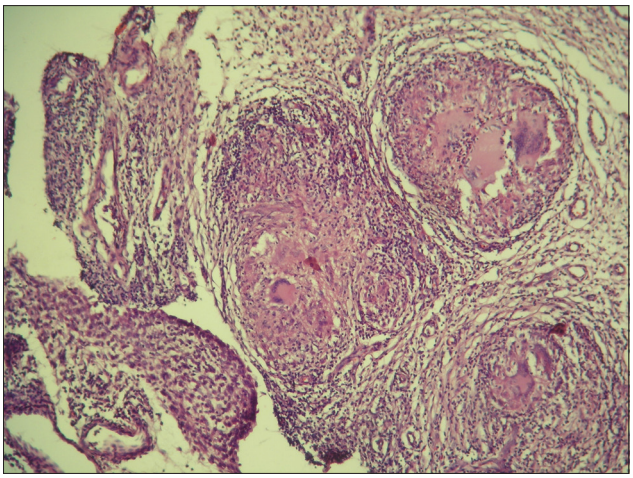

Figure 2: Histopathology slide showing epithelioid granulomas and Langhans giant cells (100×)

performed in 32 patients during the study period. The mean age of the patients was 27.13 years (range 6–42 years). The most common complaint was of swelling in the knee joint (100% patients), with pain being the second most common complaint (81.25% patients).Among the clinical signs, Joint effusion was seen in all the patients while tenderness and synovial thickening was present in 84.3 % (n= 27) and 62.5% (n= 20) patients respectively. Mean symptom duration was 8.2 months (range 1–48 months). The mean ESR in this series was found to be 43 mm/h (range 20–58 mm/h). A positive CRP (qualitative) was found in 28 out of 32 patients. All 32 samples were subjected to AMTDT, mycobacterial smear, culture, and histopathology. The comparison of results obtained by AMTDT and mycobacterial culture is shown in Table 1. With culture as gold standard, the sensitivity of AMTDT was 62.5% (Confidence Interval 25.9–89.8) and specificity was 100% (Confidence Interval 82.8–100). The positive predictive value was 100% (Confidence Interval 46.3–100) while the negative predictive value was 88.9% (Confidence Interval 69.7–97.1). With histopathology as gold standard, sensitivity was 36.36% while specificity was 95.24% with a *P* value of 0.037 (Confidence Interval 1.085–120.4). There were no false positives with AMTDT. Table 2 shows the results obtained by different investigative modalities and the percentage positivity in each method. The average time calculated in this study for performance and reporting (including transportation, processing, procedural, and reporting times) for various diagnostic modalities is shown in Table 3.

Table 1: Comparative analysis of results obtained by AMTDT vs. culture

	Culture positive	Culture negative	Total	*P* value
AMTDT positive	5	0	5	0.083
AMTDT negative	3	24	27	
Total	8	24	32	

Table 2: Results of various diagnostic investigations on synovial biopsies

Investigations	Number of positive cases	Percentages
AFB staining (direct)	0	0
AFB culture	8	66.67
BacT alert culture	8	66.67
Histopathology	11	91.67
AMTDT (Genprobe)	5	41.67
Total	12	

Table 3: Time taken to reporting by various diagnostic investigations after synovial biopsy

Investigations	Number of positive cases	Time to reporting (days)
AFB culture	8	48.6
BacT alert culture	8	22.5
Histopathology	11	6.8
AMTDT (Genprobe)	5	1.2
Total	12	

Discussion

Establishing the diagnosis of TB beyond doubt is very important when considering the cost and duration of treatment and the effects of delayed treatment including psychosocial implications.[6] Owing to the HIV pandemic, large scale immigration, and emergence of multidrug resistant strains, it is now a global problem.[17-20] Even in disease endemic countries, only clinical suspicion and imaging results are not accurate enough to diagnose and treat joint TB.[4,6] Sensitivity of most tests is very low in articular TB, as there is dilution of tubercle bacilli in synovial fluid.[6,9] Despite a wide array of the available tests like AFB culture sensitivity, AFB staining, histopathology, or even polymerase chain reaction (PCR), no single test claims to be of 100% diagnostic accuracy. In the absence of a single specific, sensitive, and rapid diagnostic method, especially in cases of early tubercular arthritis, an important priority in TB research remains to persistently find such a method

for diagnosis.[4,5,6,7,21,22] NAA tests amplify target nucleic acid regions that uniquely identify the *M. tuberculosis* complex. NAA tests for *M. tuberculosis* are broadly grouped into four types: PCR, transcription mediated amplification (AMTDT or Genprobe), ligase chain reaction, and strand displacement amplification.[23] However, all NAA methods are not as sensitive as culture, especially for the diagnosis of extrapulmonary TB.[23] Studies in the past, evaluating the role of PCR in extrapulmonary and joint TB, have reported the sensitivity variably ranging from 32 to 78%,[6,7,24-27] but no study till now has evaluated the results of AMTDT in synovial tissue samples.

Analyzing the results, out of 32 clinically suspected cases, only 12 were diagnosed to be positive for TB with either of the diagnostic tests (AMTDT, culture, and histopathology). All patients who were diagnosed with TB by any of the diagnostic modalities, i.e. AMTDT, culture, or histopathology, were started on ATT. All patients responded well to ATT. Cost constraints did not allow us to followup the positive patients with repeat AMTDT, so it being a marker for relapse or failure of therapy could not be assessed. AMTDT had a sensitivity of 62.5% and specificity of 100% with culture as the gold standard, and a sensitivity of 36.36% and specificity of 95.24% with histopathology as the gold standard. The time taken for reporting of results with AMTDT was average 1.2 days, while it was about, 22.5 days for Bactec, and 6.8 days for histopathology. O'Sullivan *et al*. reported a sensitivity of 77.3% and specificity of 98.5% for AMTDT with nonrespiratory specimens,[13] while Claudio Piersimoni *et al*.[17] reported a sensitivity of 74.3% and specificity of 100% for extrapulmonary samples. These studies were based on the collection of extrapulmonary samples from a variety of tissues including spine, lymph nodes, CSF, and gastric washing.

From a clinical standpoint, the key aspect of any new assay that detects *M. tuberculosis*, especially in an endemic area, is the assay's negative predictive value which depends on the sensitivity of the test. A test with low sensitivity like AMTDT is likely to miss out many cases in endemic areas where the disease prevalence is high. On the basis of our findings and clinical considerations, we do not recommend AMTDT to replace culture nor can it be used routinely for the diagnosis of joint TB. However, AMTDT of synovial specimens could be useful in cases in which there is a high suspicion of TB on the basis of clinical and radiological findings due to its high specificity. Hence, a positive AMTDT would provide enough certainty to initiate treatment and reduce the need for further diagnostic evaluations. However, a negative AMTDT would provide less clinical certainty because the negative predictive value would be only around 88%. There is also a definite time advantage in making a diagnosis as results with AMTDT were obtained almost within 24 h. Our study results suggests that AMTDT as well as other NAA tests for tubercle bacilli in the current form cannot replace conventional tests such as histopathology and culture in cases of tubercular arthritis. Most promising results with NAA tests in osteoarticular TB have been reported with mainly spinal samples as the concentration of bacilli is high in spinal samples leading to higher sensitivity.[6,7] These tests are good supporting tests as they have high specificity and give rapid results. So, these tests should always be used and interpreted in conjunction with conventional tests and clinical data. In such a situation, a thorough clinical examination with laboratory evidence and obtaining tissue samples in all suspected cases, preferably arthroscopically wherever possible, appears the most logical approach to deal with cases of early presenting knee joint TB.

Conclusion

In view of the low sensitivity of AMTDT, it cannot be recommended for routine use in osteoarticular TB as it is likely to miss out many cases. NAA tests, in general, are still far from being a single sensitive, specific, inexpensive, and reliable diagnostic test for joint TB, as a negative test in the presence of a clinicoradiological suspicion cannot still resolve the dilemma of the physician about tubercular versus nontubercular, unless proven in culture as well as histopathology. Arthroscopic joint evaluation and synovial biopsy specimens subjected to both histopathology and microbiological tests seem the most logical approach. In terms of diagnostic accuracy, histopathology was found to be the most economical, accurate, and time saving method.

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