Original Article

Osteoarticular tuberculosis – diagnostic solutions in a disease endemic region

Vikas Agashe, Shubhada Shenai, Ganesh Mohrir, Minal Deshmukh, Anita Bhaduri, Ramesh Deshpande, Ajita Mehta and Camilla Rodrigues

P.D. Hinduja National Hospital & Medical Research Centre, Mahim, Mumbai-400 016, India

Abstract

Background: We conducted a study of osteoarticular tuberculosis in patients from private and public settings in a disease endemic area. Our objective was to assess the role of mycobacterial culture and polymerase chain reaction (PCR) in the diagnosis of osteoarticular tuberculosis (TB) in settings where only clinical and imaging diagnosis form the basis for treatment.

Methodology: Ninety-three consecutive specimens collected from clinically suspected patients of osteoarticular TB were screened for bacterial culture, mycobacterial culture and in-house nested PCR. In addition, specimens were examined by imaging and histopathology. Ten specimens collected from patients suffering from other bone diseases were included as negative controls.

Results: Of the 93 clinically suspected TB patients, mycobacterial culture was positive for Mycobacterium tuberculosis (MTB) in 47 (51%) patients who were confirmed as definite TB cases. Of the remaining patients, 16 (17%) were diagnosed as probable, 19 (20%) as possible, and 11 (12%) as only clinically suspected TB cases. In-house nested PCR was positive in 65 (70%) cases. Fifteen patients were resistant to one or more anti-tuberculous drugs; twelve patients were multi-drug resistant, two of whom were extensively drug resistant.

Conclusion: Mycobacterial cultures using liquid media with susceptibility should form the backbone of management of osteoarticular TB. Nested PCR enhances the sensitivity if performed in addition to culture.

J Infect Dev Ctries 2009; 3(7):511-516.

Received 14 April 2009 - Accepted 24 June 2009

Introduction

Tuberculosis (TB) is a major health problem in the Indian subcontinent [1]. India accounts for nearly 20% of the total TB burden [2]. Of these, nearly 1-5% of cases have skeletal TB [3-6]. As TB is endemic in India, most orthopedic surgeons diagnose osteoarticular TB based on clinical and imaging findings only and initiate empirical anti-TB treatment. Mycobacterial culture and drug susceptibility is not performed as a routine test owing to a perceived misconception that it is difficult to achieve bacteriological diagnosis of osteoarticular TB. It is common practice to see patients being managed by unindicated, irregular and incomplete treatment schedules [7]. To address real-life issues in Mumbai, this study was initiated by members of the Bombay Orthopedic Society with the aim of assessing the role of mycobacterial culture (automated and conventional) with molecular techniques such as Polymerase Chain Reaction (PCR) in the diagnosis of osteoarticular TB in settings where clinical observation and imaging usually form the basis of diagnosis.

Materials and Methods

Members of the Bombay Orthopedic Society were requested to send representative specimens in adequate quantity from patients suspected to suffer from osteoarticular TB based on clinical and radiological (X-ray and/or MRI) features. The clinical features suggesting the diagnosis were symptoms such as pain, swelling in the joints, fever, loss of weight/appetite, cough, breathlessness, tenderness, effusion, restriction of movements, elevated ESR, and history of pulmonary TB or past TB diagnosed by either X-ray or Magnetic Resonance Imaging (MRI)/Computed Tomography (CT). Over a period of 28 months, 93 specimens were collected from suspected osteoarticular TB patients from the city public hospitals and private health care facilities, including tertiary care centers. All the specimens were analyzed for Gram stain and Acid Fast Bacilli (AFB) stain, histopathology, routine bacterial culture, and mycobacterial culture using the Mycobacteria Growth Indicator Tube (MGIT) System, plus Lowenstein and Jensen (L.J.) media and in-house nested PCR. All the specimens were divided

into two parts; one was stored at 4ºC for PCR, and the other was immediately processed as follows.:

(1) Routine culture and smears: Bacterial smears (i.e., Gram stain, Ziehl-Neelsen Carbol Fuchsin (ZNCF) stains) were made, and aerobic and anaerobic cultures were put up as per standard procedures.

(2) Mycobacterial culture: All the specimens, such as tissue/pus/synovial fluid, were inoculated into one MGIT tube along with one L.J. slant and incubated per standard procedure [8,9]. Identification of all isolated mycobacteria as *M. tuberculosis* complex or Non-tuberculous mycobacteria (NTM) was performed by the ρ-nitro benzoic acid (PNBA) assay on MGIT or ρ-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) differentiation test on the BACTEC 460 TB system. In the case of NTM mycobacteria, identification to the species level was performed using an in-house molecular test [10] and sequencing. All culture isolates were further processed for drug susceptibility testing (DST).

(3) DST: Four primary first-line and four second-line anti-TB drugs (viz. Streptomycin (SM) 2.0 μg/ml, Isoniazid (INH) 0.1 μg/ml, Rifampicin (RIF) 2.0 μg/ml, Ethambutol 2.5 μg/ml, Kanamycin (K) 5.0 μg/ml, Ethionamide (ETA) 5.0 μg/ml, ρ amino salicylic acid (PAS) 4.0 μg/ml and Ofloxacin (OF) 2.0 μg/ml) were tested using the BACTEC 460 TB system. This system works on detection of growth by measuring the amount of CO_2 generated by the metabolism of ^{14}C radiolabelled substrate (fatty acids) in the presence of different concentrations of drugs. Pyrazinamide (PZA) susceptibility testing was done by pyrazinamidase assay.

(4) Nested PCR: DNA extraction was conducted using the QIAamp DNA kit (QIAGEN), and validated in-house nested PCR was performed to amplify 245 base pair (bp) fragments of the IS6110 gene. Positive, negative and internal controls were included in every batch. The amplified products were electrophoresed on 3% agarose gel and visualized on a gel documentation system. In addition, the products of both the rounds of PCR were confirmed using a liquid hybridization assay, i.e. the DNA enzyme immunoassay (DEIA), wherein the hybridization of a single-stranded PCR product with a specific single-stranded biotinylated probe, internal to the amplified products, was carried out to validate the specificity of amplified products.

Further investigations, such as imaging (MRI and CT scan) and histopathology, were done for all these cases to compare with the microbiology results. Biopsies were performed to check osteoarticular TB, especially the presence of necrotic epitheloid granulomas with multinuclear giant cells.

All the results obtained were corroborated with clinical profiles on the basis of proforma filled out by the surgeons. The following definitions were used:

Definite TB - Patients with mycobacterial culture positive for MTB complex were considered definite TB.

Probable TB - Clinically suspected patients with biopsy and imaging (MRI/CT or Xray) suggestive of TB, but mycobacterial culture negative for MTB complex, were included in the Probable TB group.

Possible TB - Patients with clinical suspicion and imaging (MRI/CT or Xray) suggestive of TB, but not diagnostic histopathology and mycobacterial culture negative, were considered as possible TB.

Clinically suspected TB - Patients with only clinical suspicion and all other parameters such as mycobacterial culture, biopsy and imaging (MRI/CT) negative for TB or non conclusive were included in this group.

Results

In the present study, the role of mycobacterial culture and in-house nested PCR in diagnosis of osteoarticular TB was assessed by analyzing 93 clinically suspected specimens and 10 non-TB controls. All specimens were examined by five different methods (viz, AFB smear, automated MGIT culture, conventional L.J. culture, histopathology and in-house nested PCR). Results were described in table 1. The mean age of the patients included in this study was 30 years (ranging from 10 months to 80 years). Of the total, 24 (26%) patients from the pediatric age group were younger than 10 years. This was vastly different from studies done in developed countries, where the average age was 55 years and above [3,11]. The specimen types received included tissues/ tissue biopsies 64 (69%), pus 21 (22%), and synovial fluid 8 (9%). Culture recovery rate was 29/64 (45%) from tissues, 18/22 (82%) from pus, and 0% from synovial fluid.

Table 1. Results obtained by 5 different laboratory methods.

N = 103	AFB Smear		Histopath		L.J.		MGIT culture		Nested PCR	
	Pos	Pos	Pos	Neg	Pos	Neg	POS	Neg	Pos	Neg
Definite TB (47)	25	22	33	14	35	12	46	01	44	03
Probable TB (16)	06	10	16	0	0	16	01	15	14	02
Possible TB (19)	0	19	0	19	0	19	0	19	6	13
Clinically Suspected TB (11)	0	11	0	11	0	11	0	11	01	10
Non-TB controls (10)	0	10	0	10	0	10	0	10	0	10

As described in table 1, of the total 93 clinical TB specimens, 31 (33%) were AFB smear positive and 62 (67%) were smear negative. In all smear positive specimens, the number of bacilli per high-power field was less than 10, or "occasional," confirming the paucibacillary nature of these specimens. Histopathology was suggestive of TB in 49/93 (53%) specimens.

M. tuberculosis complex was cultivated in 47/93 (51%) cases. Considering culture as a gold standard, all these patients were considered as definitive TB cases. Of the 47 culture-positive specimens, 46 (98%) were positive by MGIT, whereas 35 (74%) were positive by L.J. In one (2%) specimen, the growth was observed only on L.J., and MGIT was negative (table 2). Compared to the conventional L.J. method, overall sensitivity of automated MGIT culture was 94% and specificity was 82%. Almost all the 46 clinical specimens were positive by MGIT during the first two to three weeks of incubation; however, L.J. took a longer time. Most of the specimens were positive by L.J. between four to eight weeks. An average detection time on the MGIT system was found to be 14 days, compared to 36 days by L.J.

In 16/93 (17%) patients with high clinical suspicion, imaging (MRI/CT/Xray) and biopsy were suggestive of TB; however, mycobacterial culture was negative for MTB complex. For AFB smear, 6/16 were positive. Rapidly growing non-tuberculous mycobacteria (NTM), *Mycobacterium fortuitum*, was isolated in one smear-positive patient. All these 16 cases were considered as probable TB cases.

There were 19/93 (20%) patients with clinical suspicion and imaging suggestive of TB, but all laboratory tests, histopathology, AFB smear, and mycobacterial culture were negative. All these were grouped as possible TB cases. Routine bacteriological culture analysis of all these patients showed pyogenic infections in 7/19 (37%) patients, with Gram positive *Staphylococcus aureus* isolated in six specimens, and Gram negative *Citrobacter species* was isolated in the remaining specimen.

In the present study, 11/93 (12%) patients were suspected to be suffering from TB only on a clinical basis. Imaging, histopathology, AFB smear, and mycobacterial culture results were negative in all these patients; hence they were regarded as "Non-tuberculous." Analysis of these 11 patients' specimens showed features of diverse conditions, such as inflammatory synovitis (eight), acute inflammation (three).

Ten negative control patients were negative by smear, culture, histopathology and in-house nested PCR. They included ankylosing spondylitis (one), neuroblastoma (one), lipofibromatous disease (one), stress fracture (one), cystisercosis (one), rheumatoid arthritis (four) and multiple myeloma (one).

In-house nested PCR validated 65/93 (70%) specimens as positive (table 1). Among the definitive TB group, PCR was positive in 44 of the 47 (94%) specimens. In the probable TB group, 14/16 (88%) of the specimens were positive by nested PCR; whereas 6/19 (32%) were PCR positive in the possible TB group. Among 11 patients in the clinically suspected TB group, PCR was positive in only in one (9%) specimen and was considered as false positive. All seven patients who had a pyogenic bacterial infection and one patient diagnosed with an NTM infection were negative by in-house nested PCR. All 10 negative controls were negative by in-house nested PCR. Compared to mycobacterial culture, overall sensitivity and specificity of our in-house nested PCR was 93% and 63%, respectively (table 3).

Of the 47 definitive TB patients, 30 were treatment-naïve patients and the remaining 17 had been receiving anti-tubercular treatment for varying lengths of time. In the treatment-naïve patient group, 29/30 (97%) were culture positive by MGIT for MTB complex, of whom 27 (93%) were susceptible to all the anti-TB drugs, and two were drug resistant. Of the seventeen patients receiving anti-TB treatment for various period of time, 15 (88%) were resistant to

Table 2. Comparison of culture results obtained by MGIT 960 TB system & L.J.

N = 103	MGIT Positive for MTB (46)	MGIT Negative for MTB (57)
L.J. Positive for MTB (35)	34 (72%)	01 (26%)
L.J. Negative for MTB (68)	12 (2%)	56 (0%)
Sensitivity	97%	
Specificity	82%	

one or more drugs.

Among the 15 drug-resistant cases, the resistance pattern was quite varied. Table 4 shows that 12/15 (80%) culture isolates were multi-drug resistant (MDR), i.e., resistant to INH and RIF, of which two were also XDR resistant (i.e. resistant to H, R, quinolones and amino glycosides). Forty percent (6/15) of the resistant patients were younger than 10 years in the pediatric age group.

Discussion

Infections with low numbers of bacilli are frequently seen in osteoarticular TB, which represents a clinical situation where both an early and sensitive diagnosis is of utmost importance. Proper collection from the site of the disease in a sterile leak-proof container, and rapid transportation to the laboratory, are vital for culture or PCR. In the present study, culture recovery for MTB complex was found to be 45% from tissues, 82% from pus, and 0% from synovial fluid. These results indicate that for definite diagnosis of osteoarticular TB, properly collected pus or tissue specimens from the infected area are far superior indicators over synovial fluid and should be sent to the mycobacteriology laboratory rather than only the synovial fluid.

Mycobacterial culture was found to be positive for MTB complex in 47/93 (51%) specimens. A highly enriched liquid culture media, MGIT was superior in detecting an additional 12 TB cases (receiving anti-TB treatment), which showed negative by the conventional L.J. method, thus increasing the recovery rate from 38% to 51% (table 2). Only in one (2%) specimen was the growth observed on L.J. but reported as negative on MGIT (table 2). The rate of mycobacterial culture positivity was 97% in treatment-naïve patients versus only 56% in patients receiving treatment, which indicates the importance of sending the culture before starting anti-TB treatment. Total recovery time using a liquid culture system was 14 days as compared to 36 days by the L.J. method. The average recovery time of MGIT positivity in this study was slightly higher, compared to the previous studies [12-14].

Among the probable TB group, six specimens were AFB smear, imaging, biopsy and PCR positive. In five of these six specimens, AFB culture (by both methods) was negative. All five cases had been on anti-tubercular treatment for more than six months. Culture negativity in these specimens could be due to the presence of nonviable mycobacteria in the clinical specimens. NTM (*M. fortuitum*) was isolated in the remaining patient's specimen in the MGIT 960 TB system. Disease caused by opportunist NTM, though uncommon, provides a serious diagnostic and therapeutic challenge. Most of the NTM are resistant to common anti-tubercular drugs, and they can be falsely labeled as MDRTB if not identified correctly. Hence proper identification of all cultivated mycobacteria to a species level is important. In our study we identified this NTM using an in-house molecular assay [10] and final confirmation was performed by sequencing.

Table 3. Comparison of nested PCR with Mycobacterial culture (MGIT + L.J.) results.

N = 103	Nested PCR Positive (65)	Nested PCR Negative (38)
Mycobacterial Culture Positive for MTB (47)	44 (94%)	03 (6%)
AFB Culture Negative for MTB (56)	21 (0%)	35 (0%)
Sensitivity	94%	
Specificity	63%	

Among the possible TB group where imaging findings were suggestive of TB, seven (37%) were confirmed as pyogenic arthritis by routine bacterial culture, highlighting the importance of routine culture analysis in addition to mycobacterial culture. A differential diagnosis between osteoarticular TB and pyogenic arthritis is difficult. The imaging and MRI scan can detect early joint effusions and soft tissue swelling; however, neither is specific in making the diagnosis of osteoarticular TB. In the present study, five patients were put on empirical anti-tubercular treatment based on radiology, of which only one turned out as TB. Incorrect diagnosis of TB can lead to inappropriate or unnecessary treatment.

Nested PCR was positive in 94% of the TB cases in the definitive TB group; however, there were three culture-positive PCR negative specimens (table 3). There was no PCR inhibition, as internal control was amplified in each specimen. False PCR negativity could be attributed, therefore, to the low bacterial load or to sampling error. Absence of IS6110 was excluded as a cause of false negativity as the culture from these samples indicated the presence of IS6110. Among the 11 clinically suspected cases, one was PCR positive. Upon investigation, this patient gave a past history of TB of the hip. PCR has a capacity to identify dead bacteria present in an asymptomatic person, a situation known as subclinical dissemination of MTB in absence of clinically overt disease [15,16]. This case was considered to be false positive. Nested PCR results of our study concur with the results of previous studies 12,15-18].

Table 4. Analysis of drug-resistant TB cases.

Sr. No.	Case No.	Age/Sex	Resistant Pattern	AKT Duration
1	3	17 / F	H	6 months
2	10	4 / F	S,H,R,E,K,O	1 year
3	13	2 / M	S,H,R	1 month
4	18	10 / F	R, H, ETH	6 months
5	28	11 / F	H, R, E, PAS	11\2 year
6	36	10 / F	H, S, OF	6 months
7	38	20 / F	H,R, E,S	9 months
8	57	39 / F	H, R, S	1 year
9	66	22/M	H,R,E,S	6 months
10	84	4 / F	H, R, E, S, OF	6 months
11	97	3 / M	H. R, S	6 months
12	101	9 / M	H,R, S	2 months
13	107	27 / M	H, R, S	2 months
14	118	19 / F	H,R,ETH	6 months
15	139	7 / F	H, S, E, ETH	Not started

Another important aspect of this study is the detection of resistance patterns. Of the 47 culture-positive specimens, 32 were pan susceptible, whereas 15 specimens were resistant to one or more anti-TB drug. Of the 15 resistant MTB isolates, 12 were MDR (of which 2 are XDR), one was resistant to only INH, and two were resistant to more than one drug but not MDR. None of these patients were HIV positive. In the present study, 40% of the MDR patients were between 0 to 10 years old (table 4). In the pediatric population, where compliance is assured, the high MDR rate reflects primary resistance. The association between age and osteoarticular TB is controversial.

MDRTB is rare in countries using Directly Observed Treatment Short course (DOTS). In TB endemic countries such as India, where incomplete treatment and empiric therapy (treatment without drug-susceptibility testing) are common, DOTS is certainly efficacious. Extending the DOTS for also treating extrapulmonary TB will help reduce the drug resistance burden.

Conclusions

Both routine and AFB cultures (before starting anti-TB treatment), using automation, are essential in

the accurate diagnosis of osteoarticular TB. Even in disease endemic countries, only a clinical suspicion and imaging results are not accurate enough to diagnose and treat osteoarticular TB.

Nested PCR has great potential to improve the clinicians' ability to diagnose clinically suspected TB rapidly. This will ensure early treatment for patients and prevent further transmission of the disease.

In the present study, although MGIT and PCR increased the number of definite diagnoses, they are expensive and not routinely available in TB endemic countries, except in the larger cities. In contrast, conventional culture is cost effective and was positive in 35/47 (74%) cases in the definite TB group. Minimally, AFB smear, L.J. culture and histopathology of tissue specimens can be done at a much lower cost and can increase the number of definite diagnoses.

Empirical therapy leads to needless treatment, compounds problems of drug resistance in tuberculosis, and does not accurately address the actual etiology. Drug susceptibility testing of all culture isolates should be the basis for treatment of resistant cases.

Emergence of resistance in the Indian subcontinent is a real threat. In our study, most resistant cases were in the pediatric age group and appeared to be infected with MDR bacilli.

Acknowledgements

We sincerely thank the members of the Bombay Orthopedic Society for their help in this project. We also thank the National Health and Education Society of P.D. Hinduja National Hospital & Medical Research Center for their financial support.

References

1. Cohn D, Bustreo LF, Raviglione MC (1997) Drug-resistant tuberculosis; review of the worldwide situation and the W.H.O/IULATD global surveillance project. Clin Infect Dis 24: LS121-130.
2. Paramsivan CN and Venkataraman P (2004) Drug resistance in tuberculosis in India. Review article. Indian J Med Res 120: 377-386.
3. Ruiz G, Rodrigues JG, Giierri ML, Gonzalez A (2003) Osteoarticular tuberculosis in a general hospital during the last decade. Clin Microbiol Infect 9: 919-923.
4. Al-Saleh S, Al-Arfaj A, Naddaf H, Haddad Q, Memish Z (1998) Tuberculous arthritis: a review of 27 cases. Ann Saudi Med 18: 368–369.
5. Garrido G, Gomez-Reino JJ, Fernandez-Dapica P, Palenque E, Prieto S (1988) A Review of Peripheral Tuberculous Arthritis. Sem Arthritis Reum 18: 142–9.
6. González-Gay MA, García-Porrúa C, Cereijo MJ, Rivas MJ, Ibanez D, Mayo J (1999) The clinical spectrum of osteoarticular tuberculosis in non-human immunodeficiency virus patients in a defined area of northwestern Spain (1988–97). Clin Exp Rheumatol 17: 663–669.
7. Uplekar M and Sheppard DS (1991) Treatment of tuberculosis by private general practitioners in India. Tubercle 72: 264-90.
8. Kent PT and Kubica GP (1985) Public health Mycobacteriology: a guide for level III lab. US Department of health and human services, Public health services. Center for disease control. Atlanta, 64-68.
9. Hillemann D, Richter E, Rüsch-Gerdes S (2006) Usage of the automated Mycobacteria Growth Indicator Tube System (MGIT 960) for recovery of mycobacteria from 9558 extrapulmonary specimens including urine samples. J Clin Microbiol 44: 4014-17.
10. Shenai S, Rodrigues C, Mehta A (2009) Rapid speciation of 15 clinically relevant mycobacteria with simultaneous detection of resistance to Rifampicin, Isoniazid and Streptomycin in *Mycobacterium tuberculosis* complex. Int J Infect Dis 13: 46-58.
11. Houshian S, Poulsen S, Riegels-Nielsen P (2000) Bone and joint tuberculosis in Denmark. Increase due to immigration. Acta Orthop Scand71: 312-315.
12. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, van Embden J (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis of and epidemiology. J Clin Microbiol 35: 907-14.
13. Sareen A, Khare GN, Nath G, Singh S (2006) Role of polymerase chain reaction in osteoarticular tuberculosis. Ind J Orthopaedics 40: 264-266.
14. Sun Y, Zhang Y, Lu Z (1997) Clinical study of polymerase chain reaction technique in the diagnosis of bone tuberculosis. Zhonghua Jie He He Hu Xi Zhi 20: 145-8.
15. Vohra R, Kang HS, Dogra S, Saggar RR, Sharma R (1997) Tuberculous osteomyelitis. J Bone Joint Surg Br 79: 562-566.
16. Bechnoosh A, Lieberman JM, Duke MB, Stutman S (1997). Comparison of Quantitative polymerase chain reaction, therapy for pulmonary tuberculosis. Diag Microb Infect Dis 29: 73-79.
17. Neggi SS, Khan SFB, Gupta S, Pasha ST, Kahre S, Lal S (2005) Comparison of the conventional diagnostic modalities, BACTEC culture and polymerase chain reaction test for diagnosis of tuberculosis. Ind J Med Microbiol 23: 29-33.
18. Shankar P, Manjunath N, Lakshmi R, Aditi B, Seth P, Shriniwas (1990) Identification of Mycobacterium tuberculosis by polymerase chain reaction. Lancet 335: 423–423.

Corresponding Author

Dr. Camilla Rodrigues
P. D. Hinduja National Hospital & Medical Research Centre
Veer Savarkar Marg
Mahim (West) Mumbai – 400 016 India
Phone: +91- 22 - 24447795
Fax: +91 - 22 - 2444 91 51 2318
Email: dr_crodrigues@hindujahospital.com