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ORIGINAL PAPER

The role of polymerase chain reaction in the management of osteoarticular tuberculosis

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Abstract A dependable method for the rapid diagnosis of osteoarticular tuberculosis has become increasingly important, as routine methods are neither very sensitive nor very specific. The objective of this study is to verify the reliability of polymerase chain reaction (PCR) in the diagnosis and management of osteoarticular tuberculosis. This investigation was a prospective study conducted at the Kasturba Medical College, Manipal, India. Tissue samples of 74 patients suspected of osteoarticular tuberculosis were sent for PCR and histopathologic examination. Taking histopathology as the gold standard, PCR has a sensitivity of 73.07% and a specificity of 93.75% (with 95% confidence interval [CI] 62.97; 83.17).The positive agreement between histology and PCR was 0.693, indicating good agreement. PCR showed a sensitivity of 90% with spinal samples. It has a low false positivity of 13.63%. We conclude that conventional methods are neither sensitive nor specific enough and are also time consuming. PCR is an effective method for diagnosing tuberculosis and antitubercular treatment can be started if PCR is positive, since false-positive rates are very low.

Résumé Une méthode pour le diagnostic rapide des tuberculoses osseuses articulaires voit sont importance augmenter par rapport aux méthodes de routine cependant très sensitives mais peu spécifiques. L'objectif de cette étude est de vérifier la fiabilité de la PCR (polymérase réaction en chaêne) diagnostic dans la conduite et le traitement des tuberculoses ostéo articulaires. Matériel et méthode: au cours d'une étude prospective conduite au Collège Médical Kasturba de Manipal Indes, les fragments tissulaires de 74 patients suspects de tuberculose ostéo articulaire ont été adressés, pour examen histopathologique et dosage PCR. Résultats: l'histopathologie reste le « gold standard », la PCR a une sensitivité de 73.07% et une spécificité de 93.75% (avec 95% d'intervalle de confiance CI 62.97; 83.17). La compatibilité entre histologie et la PCR est de 0.693, la PCR montre une sensitivité de 90% avec du tissu rachidien. Il existe des faux positifs (13.63%). Conclusion: les méthodes conventionnelles ne semblent ni sensitives ni spécifiques et demandent beaucoup de temps. La PCR est une méthode diagnostique de la tuberculose fiable et permet de suivre et de démarrer le traitement anti tuberculeux si la PCR est positive, les taux de faux positifs étant très bas.

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Introduction

Musculoskeletal tuberculosis accounts for 10–15% of extrapulmonary tuberculosis [22]. Any bone, joint or bursa can be infected but the spine, hip and knee are the preferred sites of infection, representing 70–80% of infections [10, 12]. Early diagnosis and the timely institution of antitubercular treatment is crucial in these cases because delay leads to irreparable damage to the joint and permanent disability of varying degree [1, 15]. The diagnosis of skeletal tuberculosis

is often delayed due to the indolent nature of the disease and, often, it can be elusive, necessitating a high degree of suspicion [8, 10, 22]. Clinical and radiological diagnosis of musculoskeletal tuberculosis are not adequately sensitive or specific, as there are many differential diagnoses for the radiological findings [6]. Conventional microbiological methods like Ziehl-Neelson staining (Z-N smear) for acid fast bacilli (AFB) and culture of Mycobacterium tuberculosis on Lowenstein Jensen media (L-J) have much lower sensitivity and specificity because of the paucibacillary nature of extrapulmonary tuberculosis [10, 24]. Also, for a positive AFB smear, 5,000–10,000 bacilli per mL are needed [5]. Most extrapulmonary specimens do not have a large concentration of bacilli. In addition, the culture of M. tuberculosis is time consuming, taking 6–8 weeks for the growth to appear [5]. So, mostly, the diagnosis of tuberculosis depends on histological evidence, which may also sometimes be inconclusive, in addition to the need for high levels of expertise.

Nucleic acid amplification (NAA) tests represent a major advance in the diagnosis of tuberculosis [4]. With the use of amplification systems, nucleic acid sequences unique to M. tuberculosis can be detected directly in clinical specimens, offering better accuracy than AFB smear and greater speed than cultures [7]. Advanced molecular methods such as polymerase chain reaction (PCR), a type of nucleic acid amplification system, have shown very promising results for the early and rapid diagnosis of the disease, due to its detection limit of 1–10 bacilli in various clinical samples [19]. It has increased the diagnostic predictability in extrapulmonary tuberculosis with paucibacillary samples [10]. This study was done to investigate the utility of PCR for the early diagnosis of bone and joint tuberculosis and its comparative evaluation with histopathological examination. We also tried to compare its utility in the diagnosis of spinal and extraspinal tuberculosis (hip, knee etc.).

Materials and methods

Study design and setting

This was a prospective study carried out from November 2003 to May 2007 at Kasturba Hospital, Manipal, India, involving the Department of Orthopaedic Surgery and the Department of Microbiology of Kasturba Medical College, Manipal, India.

Selection of patients

Patients were selected depending on the clinical and radiological suspicion of tubercular involvement of bone or joints.

Exclusion criteria

All of those patients who were already diagnosed for osteoarticular tuberculosis and the patients who were on antitubercular treatment were excluded.

Sample size

During this time period, 74 patients suspected of osteoarticular tuberculosis involving bone or joints were included in the study.

Collection of samples

The samples were collected under strict aseptic precautions in the operation theatre. They included synovial fluid, pus from the diseased part and tissues, such as synovium or vertebral bone (closed or open biopsy). All of the samples were subjected to PCR and histopathological examination. Tissue samples for PCR were sent in normal saline and for histopathology in 10% formalin. All of the samples were kept at 4°C before proceeding for PCR. All of the samples for PCR processing were handled by the same senior technician in the Department of Microbiology (SR) under the guidance of the senior microbiologist (KC). All steps of the PCR were carried out in separate rooms to minimise the chance of the carry-over of templates.

All histopathology slides were reviewed by two senior pathologists after processing.

Processing of samples for PCR

Pus samples were decontaminated with 4%NaOH for 10 min and then centrifuged at 6,000 rpm for 20 min. The supernatant was discarded and an equal amount of phosphate buffer was added to the sediment. All of the biopsies (tissue or bone) were homogenised in pestle and mortar, centrifuged at 6,000 rpm for 10 min and, to the pellet, 3 ml of Tris buffer was added. Synovial fluid was centrifuged at 6,000 rpm for 10 min and, to the pellet, 3 ml of Tris buffer was added.

DNA extraction

All of the processed samples (decontaminated pus, synovial fluid, homogenised tissues) were centrifuged again at 6,000 rpm for 10 min and, to the resultant pellet, 250 μl of lysis buffer I and 20 μl of proteinase K was added (provided in the kit from Bangalore Genei, Bangalore, India). After mixing by vortexing, all of the samples were kept in a dry bath at 90°C for 20–25 min and then centrifuged at 10,000 rpm for 10 min. To 200 μl of supernatant, 200 μl of lysis buffer II (containing internal control at a concentration of 10 μl/ml) was added in 1.5-ml eppendorf tube (Axygen Scientific, Mahalasa Agency,

Mangalore, India) and incubated at 70°C for 10 min. After that, 200 μl of 96–100% ethanol was added and mixed by vortexing. This mixture was added to a spin column placed in a 2-ml collection tube and centrifuged at 6,000 rpm for 3 min. The spin column was then kept in a new 2-ml collection tube and washed twice with wash buffer (provided in the kit) and a final centrifugation was done at 14,000 rpm for 2 min to ensure complete removal of the wash buffer. Then, the spin column was kept in a 1.5-ml tube and 100 μl of pre-warmed (50°C) elution buffer (provided in the kit) was added. After incubating at room temperature for 5 min, it was centrifuged at 10,000 rpm for 2 min to elute the DNA. The DNA samples were kept at −20°C for further use.

Polymerisation of DNA

Two-step nested PCR was performed by a commercial kit method from Genei Bangalore (India) for IS6110 of M. tuberculosis.

Analysis of amplified products

Amplified DNA was electrophoresed using 1.5% agarose gel at 120 v for 1 h and the resultant bands were interpreted by UV transillumination. The product of 123 bp was indicative of infection with M. tuberculosis and the amplified product of 340 bp was showing internal control DNA.

Histopathological examination

All of the tissues received for histopathology were fixed in 10% formalin, embedded in paraffin, cut to 5-μm-thick sections and stained with hematoxylin-eosin (H&E) and Gabbet's stain before microscopic examination. The presence of typical caseating granulomas and/or Langhans' giant cells on H&E staining and the identification of acid fast bacilli on Gabbet's staining were considered as proof of tuberculosis.

Results

PCR and histopathological examination were performed on a total of 74 patients. Of these 74 samples, 22 were from the spinal region for patients suspected for Pott's spine and 52 were from various bones and joints, mostly from the knee and hip (synovium, synovial fluid, bone biopsy or pus). Taking histopathology as the gold standard, the overall sensitivity and specificity observed for PCR was 73.07% (with 95% confidence interval [CI] 62.97; 83.17) and 93.75% (with 95% CI 88.27; 99.26), respectively, when all of the samples were taken together. The positive predictive value was 86.36% (19 out of 22) and the negative predictive value for the test was 86.53% (45 out of 52).The overall false positivity rate was 13.63% (3 out of 22) and the overall false-negative rate was 26.92% (7 out of 26). Positive agreement between histopathology and PCR was 0.693 ($p< 0.05$), indicating good agreement. The comparison of results obtained by PCR and histopathological examination for all of the samples taken together is shown in Table 1. When the observations were made only for spinal samples, the results were different (Table 2). The sensitivity was 90% (9 out of 10) and the specificity was 100% (12 out of 12). The positive predictive value was 100% (9 out of 9) and the negative predictive value was 92.30% (12 out of 13). The false positivity rate was 0% (0 out of 9) and the false negativity rate was 10% (1 out of 10). Positive agreement between histopathology and PCR was 0.908 ($p<0.05$), indicating strong agreement. The observations for non-spinal samples were different (Table 3). The sensitivity was 62.5% (10 out of 16) and the specificity was 91.66% (33 out of 36). The positive predictive value was 76.92% (10 out of 13) and the negative predictive value was 84.61% (33 out of 39). The false positivity rate was 23.07% (3 out of 13) and the false negativity rate was 37.5% (6 out of 16). Positive agreement between histopathology and PCR was 0.571 ($p<0.05$), indicating fair agreement.

Table 1 Comparison of the results obtained by polymerase chain reaction (PCR) and histopathological examination for all of the samples taken together (n=74)

	Histopathology positive	Histopathology negative
PCR positive	19	3
PCR negative	7	45

Table 2 Comparison of the results obtained by PCR and histopathological examination for the samples taken from the spine (n=22)

	Histopathology positive	Histopathology negative
PCR positive	9	0
PCR negative	1	12

Discussion

Osteoarticular tuberculosis is a major problem in the developing world and it is one of the major causes of

Table 3 Comparison of the results obtained by PCR and histopathological examination for the samples of various synovial tissues and synovial fluid (n=52)

	Histopathology positive	Histopathology negative
PCR positive	10	3
PCR negative	6	33

osteomyelitis. In the event of HIV, it is now re-emerging [2, 11]. The spine is the site most commonly affected with tuberculosis, followed by the hip and the knee [23, 25]. The diagnosis of osteoarticular tuberculosis is often delayed, on average, by 16 to 19 months [8]. Routine blood and radiological investigations may not be very helpful in the diagnosis of early osteoarticular tuberculosis [3, 13, 25]. Z-N smear examination and traditional culture (Lowenstein Jenson Media) methods are also not very sensitive and often show low positive or negative results [10, 24]. Even the recently developed radiometric Bactec culture method takes an average time of 23.2 to 32.6 days and its sensitivity is low [15]. However, its high cost and the need for the safe disposal of the radioactive waste preclude its use in peripheral laboratories. The diagnosis of tuberculosis is perforce based on histopathological examination, which calls for professional expertise. It also takes about 2–3 weeks for the report to come back, depending upon the type of sample. The presence of classical caseating tubercle granulomas is a must for establishing the diagnosis of tuberculosis. But the tubercle may be absent in many samples [16]. The dilemma, then, is between clinical suspicion and confirmatory evidence. PCR is now an established method of diagnosing tuberculosis in a rapid manner. It can detect tubercular bacilli, even if they are present in extremely low quantities, as low as 10 fg. Further, the diagnosis can be established within 24 h [15]. If the diagnosis of tuberculosis can be made rapidly, the timely institution of antitubercular treatment can prevent further joint damage and disability.

Analysing the results, it is apparent that the sensitivity of PCR is quite high in spinal samples as compared to non-spinal samples; 90% and 62.5%, respectively. The overall sensitivity of PCR was 70.83% in this study. Various studies have reported sensitivity ranging from 61% to 83% [15, 18, 20, 21]. The low sensitivity can be explained due to the dilution of tubercle bacilli in non-spinal samples, such as synovial fluid and synovial tissues. Bone samples from the spine have higher concentrations of bacilli and, consequently, yield higher sensitivity. Similar observations were made by Negi et al. [15]. The trend of high false negativity in non-spinal samples indicates that, if PCR is negative in such samples, one should always peruse the histopathology slides more carefully for any evidence of tuberculosis. Three of 22 (13.63%) cases yielded false-positive results (PCR positive and histopathology negative). These were the cases where histopathological evidence could not be established due to absent caseating granulomas and were not the real false-positives. Spatial separation for all steps of PCR reduced the incidence of false positivity. Further, the patients had strong clinical and radiological indication for tuberculosis and responded well to anti-tuberculous therapy. Patients with positive PCR and negative biopsy should be given a trial of treatment for tuberculosis. As the specificity of PCR is high (93.75%), a negative PCR would rule out tuberculosis if biopsy is also negative. A positive biopsy would call for antitubercular treatment, irrespective of PCR status. If PCR is negative, further decisions would be based on the histopathological examination.

Establishing the diagnosis of tuberculosis beyond doubt is very important when considering the cost and duration of treatment and the effects of delayed treatment [14]. Moreover, it has its economic and psychosocial implications in the developing world [9, 17]. False positivity in PCR can be minimised by the adequate training of personnel in molecular methods and preventing laboratory-introduced contamination.

Conclusions

Polymerase chain reaction (PCR) is a rapid method of diagnosing extrapulmonary tuberculosis with high sensitivity and specificity. Its sensitivity is higher in spinal samples. Hence, in samples from areas other than the spine (bone), histopathology correlation is advisable. Low false positivity rates in PCR dictate that the PCR-positive–biopsy-negative patients should be given a trial of antitubercular treatment.

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