Original Article

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# Evaluation of the Diagnostic Value of Mycobacterium Tuberculosis DNA Recognition by Polymerase Chain Reaction (PCR) on Bone Tuberculosis

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## Abstract

**Background:** This study aimed at evaluating the function of polymerase chain reaction (PCR) in the identification of spinal tuberculosis post two weeks of preoperative anti-tuberculosis cure and to evaluate PCR to the Lowenstein - Jensen Culture (LJC) and histopathological examination (HPE) methods. **Subjects and Methods:** All of the tissues obtained for histopathology were fixed in 10% formalin, embedded in paraffin, cut to 5-$\mu$m-thick sections and stained with hematoxylin-eosin (H&E) and Gabbet's stain before microscopic examination. The sample collection was executed in sterile situations. Joint fluid, pus or tissue samples were composed and ground. **Results:** Results demonstrated that the PCR approach had 80.9% sensitivity, 95.2% specificity and 87.3% accuracy respectively. ELISA for TB-SA antibody had 51.8% sensitivity, 82.4% specificity and 66.4% accuracy respectively. The bacterial culture had 80.9% sensitivity, 68.1% specificity and 43.2% accuracy respectively. **Conclusion :** Polymerase chain reaction (PCR) is a speedy technique for identifying extrapulmonary TB with high sensitivity and specificity.

**Keywords:** Polymerase Chain Reaction, Mycobacterium Tuberculosis, DNA, ELISA

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## Introduction

Tuberculosis (TB) is sourced by an assortment of species of mycobacteria among which Mycobacterium tuberculosis (MTB) is the most recurrent which is a slow mounting facultative intracellular parasite.[1,2]

Identification in an early stage of the ailment is of dominant significance for management instigation, with unswerving results for individual illness prevention and, consequently, for public health inventiveness intended at the deterrence of tuberculosis diffusion. Immediate cure of spinal tuberculosis with prescribed medicines is imperative in illness administration. Essentially, well-timed handling is characteristically commenced diagnosis on clinical and radiological substantiation with no waiting for culture outcomes, particularly in regions prevalent for tuberculosis and developing nations.

Usually, TB is identified by conformist techniques like sputum smear microscopy, chest radiographic findings and culture studies.[3] The prevailing methods stay unproductive due to confines in low mycobacterium levels and time taking practices.[4] precise and timely diagnosis of tuberculosis is significant for an efficient cure and appropriate handling.

Substantiation of TB by positive culture often takes three to six weeks. Even before the accessibility of culture outcomes, the sufferers may have a clinical decline, which could be connected to a substitute non TB contagion, side effects of anti-tuberculosis cure, anti-tuberculosis drug conflict, or a paradoxical retort. The consequences of TB PCR testing can facilitate to intensify the pronouncement making procedure concerned in the analysis of TB, so that early on anti-tuberculosis cure can be started. The laboratory presentation of both commercial and in house TB PCR in the analysis of TB has been expansively assessed.[5–9]

MT can be sent out to a variety of organs like skin, peritoneal, lung and bones via blood-borne or lymph circulation.[10] Bone TB is one disparaging laceration grounded by the blood-borne incursion of MT into bone or joint tissues and regularly happens in the spine, hip, feet, elbow, or hand to influence loading of bone, joint tissues and limb motility.[11] Bone TB is frequently associated with a low fever, lassitude,

night sweat, lower appetite and thinning, plus joint swelling, pain, atrophy and/or dysfunction, causing major issues for the sufferer.[12]The solution for bone TB embraces early identification and opportune cure to lessen joint structural injure and holds back efficient hurt.[13] Though due to poorer specificity in the untimely clinical demonstration, the well-timed identification of bone TB is comparatively complicated and needs manifold approaches coiffure history, body sings, auxiliary assays and MT culture.[14,15] These methods, however, have a relatively lower sensitivity and specificity.[16] This study thus tested MT DNA using polymerase chain response (PCR) assessment to examine its insinuation for bone TB identification.

## Subjects and Methods

### Study design and setting

This was a potential study performed from September 2018 to October 2020 at Saraswathi Institute of Medical Sciences, Anwarpur, Pillkhuwa, Distt. Hapur India, concerning the Department of Orthopedic and the Department of T.B. & Chest. This work gained signed informed consent from all participants and was permitted by the Ethics Committee.

A total of 110 samples were collected from bone tuberculosis patients who were admitted to the hospital including 68 males and 42 females (aging between 25-70 years).

Ninety non-bone tuberculosis samples (control group) were collected, including 65 males and 30 females, with ages between 24 and 66 years.

### Inclusive criteria

Typical TB pathology modifies under assay or during surgery; visible MT after bacterial culture; typical clinical symptoms, body signs and imaging features; effective anti-TB treatment. A patient can be diagnosed with any one item of those abovementioned features.

### Exclusion criteria

Accompanied with other infectious diseases, malignant tumors, severe diabetes, kidney/liver disease, pulmonary fibrosis, bone metabolic disorder, systemic immune disease or any complication of cancer.

### Histopathological examination

All of the tissues obtained for histopathology were fixed in 10% formalin, embedded in paraffin, cut to 5-$\mu$m-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.

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 $\mu$l of lysis buffer I and 20 $\mu$l of proteinase K was added (provided in the kit from Bangalore Genei, Bangalore, India). The DNA samples were kept at −20°C for further use.

The sample collection was executed in sterile situations. Joint fluid, pus or tissue samples were composed and ground. After adding lysis buffer, tissues were homogenized and centrifuged. The tissue supernatant was used as the template. Joint fluid samples were frequently centrifuged for the objective of collecting the supernatant. All of the samples were subjected to PCR and histopathological assessment. 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 minimize the chance of the carry-over of templates. All histopathology slides were reviewed by two senior pathologists after processing.

### The polymerisation of DNA

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

### Statistical Analysis

SPSS 19.0 statistical software was used for analysis. Enumeration data were tested by the chi-square method. A statistical significance was defined when $P < 0.05$.

## Results

ELISA was used to test for the TB-SA antibody, and 57 positive cases were found in the bone tuberculosis group (53 negative cases, positive rate = 51.8%). In the control group, there were 16 positive cases and 74 negative cases (positive rate = 17.7%). In MT culture, there were 23 positive and 87 negative cases in the bone tuberculosis group (positive rate = 20.9%), and 29 positive and 61 negative cases in the control group (positive rate = 32.2%). PCR assay thus had a significantly higher positive rate of MT DNA than that of ELISA or bacterial culture approach. ELISA also had a higher positive rate of bone tuberculosis than bacterial culture. In the control group, the DNA positive rate was significant than ELISA or bacterial culture approach, whilst ELISA had a lower positive rate than the culture method ($P < 0.05$, [Table 1]).

Sensitivity and specificity analysis were performed among the PCR assays for MT DNA, ELISA for TB-SA antibody and MT culture. Results demonstrated that the PCR approach had 80.9% sensitivity, 95.2% specificity and 87.3% accuracy

**Table 1: Comparison of PCR for MT DNA, ELISA for TB-SA and MT culture**

| Approach | Bone tuberculosis | | | Control | | |
|---|---|---|---|---|---|---|
| | Positive cases | Negative | Rate (%) | Positive cases | Negative | Rate (%) |
| PCR for MT DNA | 89 | 21 | 80.9*,# | 3 | 87 | 3.3*,# |
| ELISA for TB-SA antibody | 57 | 53 | 51.8* | 16 | 74 | 17.7* |
| MT culture | 23 | 87 | 20.9 | 29 | 61 | 32.2 |

Note: *, P < 0.05 compared to MT culture; #, P < 0.05 compared to ELISA

respectively. ELISA for TB-SA antibody had 51.8% sensitivity, 82.4% specificity and 66.4% accuracy respectively. The bacterial culture had 80.9% sensitivity, 68.1% specificity and 43.2% accuracy respectively. The PCR approach displayed significant advantages over the other two approaches (P < 0.05, Table 2).

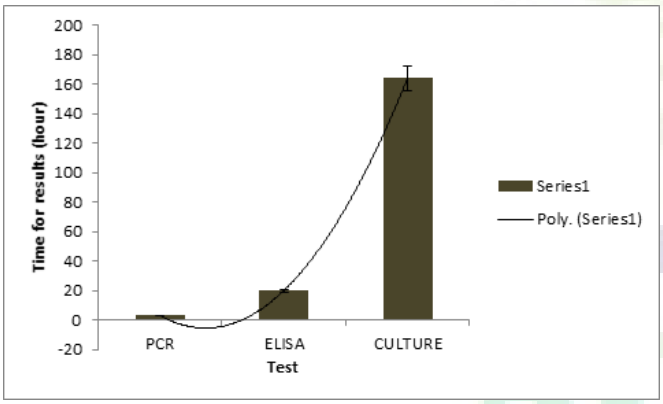


**Figure 1: Comparison of time consumption among the three approaches**

## Discussion

Bone TB has a subtle onset which develops gradually with the lack of typical indications at an early stage. Imaging processes like X-ray thus may not be capable to recognize the subsistence of early lesions. Though CT has a moderately elevated resolution for bone tissues, it has low sensitivity for sensing provocative alterations of synovial tissues and joint cavity fluids. Consequently, an inclusive diagnosis counting medical indications and lab outcomes like erythrocyte sedimentation are essential for the diagnosis. The complexity of establishing a diagnosis recurrently roots to ailment succession and in some cases, severe provocative injury and dysfunction of bone-joint structure. The study for the early and fast diagnostic methods is significant for deterrence and diagnosis of bone TB.[17,18]

The spine is the spot most frequently affected with tuberculosis, followed by the hip and the knee.[19,20] The diagnosis of osteoarticular tuberculosis is often delayed, on average, by 16 to 19 months.[21] Routine blood and radiological investigations may not be very handy in the diagnosis of early osteoarticular tuberculosis.[20,22] Z-N smear assessment and traditional culture (Lowenstein Jenson Media) methods are also not very receptive and frequently demonstrate squat affirmative or negative results.[23] Even the lately developed radiometric Bactec culture method takes a standard time of 23.2 to 32.6 days and its sensitivity is low.[24]

This study exploited the PCR approach for intensifying 245 bp length DNA portion as the template, considerably uplifting the positive rate of bone tuberculosis assay and the test sensitivity and specificity, with less time and higher accurateness. Consequently, the PCR approach presents clinical significance for early diagnosis of bone TB. PCR approach for MT DNA has abundant benefits in terms of high sensitivity, specificity, promptness, and ease of exploitation, all of which are beneficial over ELISA for TB-SA antibody and MT culture approaches. PCR assay thus offers a novel process for early, rapid and precise identification of bone TB, and has significant clinical inference.

Current progress in the molecular diagnosis of TB linking the uncovering of bacterial mRNA encoding the ubiquitous 85B antigens in viable M tuberculosis by reverse transcriptase PCR and reverse transcriptase strand dislodgment magnification assessment may conquer the above complicatedness.[18–20] Because the mRNA is more rapidly shattered in the cells than rRNA or genomic DNA, having a half-life of only a few minutes, it can distinguish between viable and nonviable M TB. Therefore, it may become helpful for the diagnosis of active TB and supply a tool to monitor the therapeutic effectiveness.

Setting up the diagnosis of TB beyond doubt is very vital when taking into consideration the cost and period of handling and the effects of delayed treatment.[25] Moreover, it has its economic and psychosocial implications in the developing world.[26,27] Counterfeit positivity in PCR can be minimized

**Table 2: Sensitivity and specificity analysis among PCR, ELISA, and culture**

| Test method | Sensitivity | Specificity | Accuracy |
|---|---|---|---|
| PCR for MT DNA | 80.9*,# | 95.2*,# | 87.3*,# |
| ELISA for TB-SA antibody | 51.8* | 82.4* | 66.4* |
| MT culture | 20.9 | 68.1 | 42.2 |

Note: *, P < 0.05 compared to MT culture; #, P < 0.05 compared to ELISA

by the ample training of personnel in molecular methods and putting off laboratory-introduced contagion.

## Conclusion

PCR method for MT DNA has plentiful rewards in terms of high sensitivity, specificity, promptness, and effortlessness of treatment, all of which are beneficial over ELISA for TB-SA antibody and MT culture approach. PCR assay thus supplies a novel method for early, rapid and correct identification of bone TB, and has imperative medical insinuation. Polymerase chain reaction (PCR) is a speedy technique for identifying extrapulmonary TB with high sensitivity and specificity. TB PCR is a rapid and reliable method in the analysis of TB, which allowed the early beginning of anti TB cure in more than 50% of the studied sufferers in our legion. Though, because of the comparatively low sensitivity of TB PCR, clinical judgment remains the final assessment in the management of TB.

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