

PCR: A FAST DIAGNOSTIC INVESTIGATION FOR SPINAL TUBERCULOSIS

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Abstract :

PCR, as a new faster diagnostic modality for tuberculosis is slowly picking up in our country where nearly six million cases of tuberculosis exists, of which 3% are skeletal tuberculosis. Confirmation of diagnosis by traditional methods takes around couple of weeks and requires a high bacteriological load in the sample.

We are presenting a spinal tuberculosis case where preoperative sample from the surgical empyema proved sufficient for definitive diagnosis of tuberculosis using PCR against hsp65 gene with nucleotide sequence specific for *Mycobacterium tuberculosis*.

With this case report we have been able to bring about the advantages of PCR i.e. high specificity, early diagnosis and the ease of operations in PCR as compared to other traditional diagnostic modalities for tuberculosis.

Keywords : *Mycobacterium tuberculosis*, hsp65 gene

Introduction:

Tuberculosis is still a highly prevalent disease in India with nearly six million radiological proven cases, of which at least 3% are skeletal tuberculosis.¹ The diagnosis of tuberculosis is based on compatible history, clinical features, radiological features, positive Mantoux test, demonstration of acid-fast bacilli (AFB) in smear (from intra-operative tissue), culture of fluids for *Mycobacterium tuberculosis* and response to treatment.² However, the clinical findings and fluid hematology/biochemistry may be variable and Mantoux test may be falsely negative. AFB isolation from fluid is very difficult and requires high concentration of the bacteria to the tune of 1000-10000 bacilli/mm³. To culture the bacteria is a tedious job and takes a minimum of 2 weeks to get a preliminary report and

at least 6-12 weeks for the conclusive report. Under such circumstances, the anti-tuberculous therapy is started empirically, waiting for clinical improvement. Hence it would be beneficial to the clinician if a diagnostic

investigation is available that is both sensitive and specific to tuberculous bacilli.

Polymerase chain reaction (PCR) is a new diagnostic modality which amplifies DNA specific to *Mycobacterium tuberculosis* PCR is considered to have high sensitivity and specificity and provides results within 48hrs.³ Based on this significant investigation we present a case of tuberculosis spine diagnosed using PCR from fluid obtained by surgical empyema secondary to tuberculosis

Case History:

42 yr old male, presented to us with 7 month history of back ache. 2 month following onset of progressive weakness in his lower limbs to the present state of complete paralysis. He also had developed breathing difficulty in the last 3 weeks. On examination patient was found to be paraplegic and an angular kyphosis at thoracic vertebrae 5-7. Respiratory system examination revealed decreased air entry on the right side, with diffuse crepitations. With a working diagnosis of tuberculosis, patient was investigated as detailed in table 1

Radiological investigations included plain radiographs of

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spine and chest, CT of spine and MRI of spine and chest.

Both chest radiograph and MRI confirmed the presence of an abscess in D4-D6 level, and also the presence of pleural collection on right side. (Figures 1, 2)

As investigations strongly pointed towards tuberculosis, and the presence of pleural fluid providing a ready sample for microbiological investigation, pleural fluid aspiration was done. About 5ml of pus was aspirated (from right 6th intercostal space) which was sent for grams staining, cell type, cell count and culture. As the diagnosis of tuberculosis was to be confirmed, a sample was also sent for PCR. Mycobacterial culture was deferred as it would take at least 4-6 weeks for the report.

Cell type, and cell count suggested chronic inflammatory picture. PCR was diagnostic of presence of hsp65 receptor gene specific for *Mycobacterium tuberculosis*. (Figure 5)

Based on this confirmation, and a negative growth in culture in the initial 24hrs, evacuation, debridement, and fusion at D4-D5 level was done through transthoracic approach. At the same time evacuation of the surgical empyema was also done and about 250ml of pus was drained. (Figure 3)

Postoperatively patient was started on anti tubercular regimen, and patient respiratory symptoms improved significantly. The neurological status remained status quo.

Intra-operative samples were sent for culture of mycobacterium tuberculosis and repeat PCR. 2 weeks preliminary report showed no growth. PCR was again positive for hsp 65 gene.

At the end of 3 months follow-up, the patient's general condition improved significantly. He developed paraesthesia in his lower limb. X-ray showed consolidation of the fusion. ESR had decreased to 45mm/Hr. (Figure 4)

Discussion:

Polymerase chain reaction is an excellent diagnostic tool with good sensitivity and specificity.⁴ In extra pulmonary tuberculosis, where pre operative sample collection for any

microbiological diagnostic procedure is almost not possible, PCR as a diagnostic tool is very useful. Sample required for PCR is relatively small and can be from any part of the diseased tissue. Even as little as 1 to 10 bacilli in the sample are sufficient for diagnosis.⁴ AFB smears require 10⁵ AFB/ml of sputum for recognition by direct microscopy, culture detects as few as 10 to 100 CFU/ml of sputum in the case of pulmonary tuberculosis.

Usually, a diagnosis of Osteoarticular tuberculosis depends on microbiological testing, such as smear or culture, and histological tissue examination. A molecular biology-based method capable of detecting and identifying specific pathogens in tissues would be very useful, because not only could such a method sensitively detect and specifically identify the pathogens present, but it could also reduce the time required for testing compared the times required for microbiological identification procedures.⁵

PCR was used to amplify hsp65 DNA(604 base protein). Hsp65 was found to be very useful in differentiating the various *Mycobacterium* species. One advantage of hsp65 Duplex PCR system is the ease of operations.⁶ It can be used in a general clinical setting, negating the requirement of costly lab equipment. Hsp65 amplification kits are available nowadays, which can be used on sample and results can be given in 1-2 days.⁷

In this case report we have used the pleural fluid sample that was obtained by a bedside procedure. With this sample, that showed a very low bacterial load, we have been able to obtain a positive identification for *Mycobacterium tuberculosis*. And using hsp65 has made the diagnosis more specific for *Mycobacterium tuberculosis*. The speed with which the diagnosis was confirmed in relation to standard culture techniques is a huge advantage.

As the diagnosis is confirmed, there was no need for other investigative modalities, like culture or smear. The disadvantage though is that PCR cannot be a prognostic indicator. The availability of a prepackaged kit with instructions, quality- controlled, standardized reagents,

and the new AmpErase system to prevent carryover contamination reduces the technical demands for performing PCR. Also the specificity in identifying individual species of bacteria by PCR scores over culture methods, where the risk of contamination is always present. The requirement of maintaining ideal conditions for culture of tubercle bacilli is also avoided. PCR is also cost effective. Though the individual kits are costly, when compared to culture costs, its considerably less.

Table 1: Pre-operative blood investigation of the patient.

Blood Investigations:	
Hemoglobin	9.7gm%
Total Count	27000/cmm
Differential Count	
Neutrophils	43%
Lymphocytes	51%
Eosinophils	2%
Monocytes	4%
ESR*	105mm/hr
CRP**	48mg/dl
Random Sugar	110mg/dl
Blood Urea	43mg/dl
Serum Creatinine	1.3mg/dl



Figure 1: X-ray chest AP (pre-op) showing empyema thoracis on right side

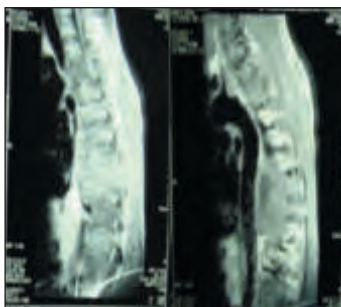


Figure 2: MRI showing involvement of D8 vertebrae

Conclusion:

Using PCR as a diagnostic tool for extrapulmonary tuberculosis has changed the management of this disease, but the disadvantage being that this sample for diagnosis had to be obtained intraoperatively. This report presents an opportunity of preoperative diagnosis of spinal tuberculosis using sample obtained from associated empyema thoracis.



Figure 3: Post-op x-ray showing drainage of empyema with ICD in situ.

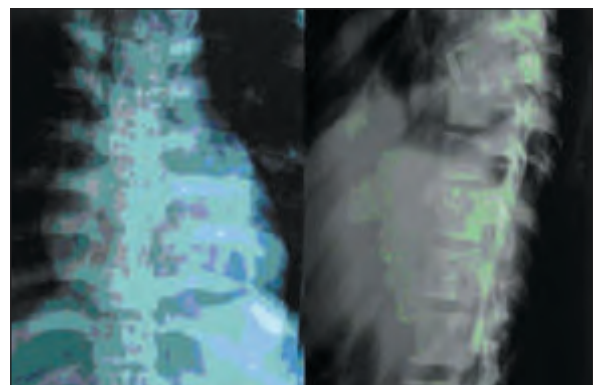
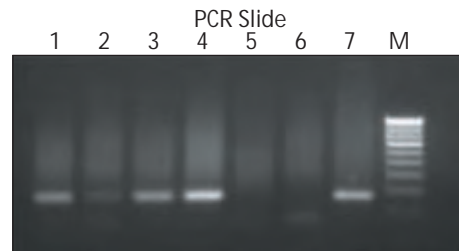


Figure 4: 3 months follow-up showing fusion with graft in place.



Lane 1,2,3,4 : DNA samples of spinal fluid
 Lane 5,6 : Negative control
 Lane 7 : Positive control (150bp).
 Lane M : Molecular weight marker (100bp)
 Positive for Mycobacterium tuberculosis by Nested PCR targeting hsp65 gene

Figure 5: Detection of hsp65 gene of M. tuberculosis using nested PCR

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