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DIAGNOSIS OF EXTRA PULMONARY TUBERCULOSIS BY REAL TIME PCR AND COMPARISON WITH CONVENTIONAL DIAGNOSTIC METHODS

Microbiology

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ABSTRACT

Introduction : Tuberculosis is a major global public health problem. Among that Extra Pulmonary Tuberculosis (EPTB) accounts for approximately 15-20% of the tuberculosis cases. Tuberculosis can involve any organ system in the body though pulmonary tuberculosis is the most common presentation. Extra pulmonary Tuberculosis is also an important clinical problem.

Materials and methods: Various body fluids submitted for confirmation of diagnosis of TB were prospectively studied. These include CSF, pericardial fluid, pleural fluid, peritoneal fluid, peritoneal dialysis fluid, synovial fluid, Urine and Pus.

Results: A total number of 200 samples were received during study period and they were also tested for ZN staining and culture on LJ Medium for the detection of Mycobacterium tuberculosis. Of these, 29 were positive for tuberculosis. RT PCR detected maximum number of positives (23), followed by culture (16) and the smear (2). PCR and culture positives were 10 and smear and culture positives were 2 and all positives were 1(pus).

Conclusion : PCR assay is highly sensitive and specific tool available to date for the diagnosis of tuberculosis in all types of specimens obtained from patients with a clinical suspicion of extra-pulmonary and can be reliably used for rapid identification of tuberculosis.

KEYWORDS

Extra Pulmonary Tuberculosis, PCR assay, *Mycobacterium tuberculosis*

INTRODUCTION :

Tuberculosis is a disease of great antiquity. In the past, tuberculosis has been referred to as captain of death.[1] Tuberculosis remains a major global public health problem. According to World health organization (WHO) report, more than 80% of all TB patients live in sub-Saharan Africa and Asia. India ranks first in the list of 22 high burden countries.[2] Over 70% of cases occur in the economically productive age group 15 - 45 years. A patient with infection of pulmonary Tuberculosis can infect 10-15 persons per year.[3]

Of particular concern is the increasing number of multidrug resistant tuberculosis cases and extensively drug resistant tuberculosis cases.[4] According to WHO, one of fourth tuberculosis death is HIV related, there were 4,56,000 death and an estimated 1.37 million new tuberculosis cases among HIV infected individuals. Apart from *M.tuberculosis* complex, there is growing number of Non Tuberculosis Mycobacterium (NTM) species, some of which are sources of important diseases in humans.[5]

Paleopathology studies show that *Mycobacterium tuberculosis* complex was detected even in Egyptian mummies by making use of DNA and HPLC analysis.[6] The earliest classical descriptions of tuberculosis in Greek literature date back to writing by Hippocrates. Tuberculosis didn't become a major problem until the industrial revolution, when crowded living conditions favored its spread. In the 17th &18th centuries, tuberculosis cause of all deaths in Europe.[7] The bacillus causing actual disease is *Mycobacterium tuberculosis,* was identified and described by the German physician Robert Koch on 24th march, 1882 at Berlin patho physiological society. In 1886, Edward Livingstone, a physician who recovered from tuberculosis started sanatorium treatment in New York.

Tuberculosis can involve any organ system in the body though pulmonary tuberculosis is the most common presentation. Extra pulmonary tuberculosis (EPTB) is also an important clinical problem.[8,9,10] The EPTB has been used to describe isolated occurrence of tuberculosis at body sites others than the lung. In HIV positive patients, EPTB accounts for more than 50% of all cases of tuberculosis.[11,12] It estimated that EPTB constitutes 15-20% of tuberculosis cases in general practice among HIV- negative adults in Indian.[13] Increase in tuberculosis incidence has been observed in recent years, coupled with emergence of Mycobacterial strains resistant to most effective drugs. These trends have prompted the search for an efficacious and rapid technique for detection of *Mycobacterium tuberculosis*.

Mycobacteria may not be suspected as the causative agent of an extra pulmonary disease because the chest X-ray or the tuberculosis test is negative or both. However, based on clinical symptoms and because Mycobacteria can infect almost any organ in the body, the laboratory should expect to receive a variety of extra pulmonary specimens such as body fluids, surgically excised tissues, aspirates or draining pus and urine.

Extra pulmonary specimens are divided in to two groups based on the site and mode of collection and the extent of contamination. Aseptically collected specimens, usually free from others microorganisms (sterile)- fluids like spinal, pleural, pericardial, synovial, ascetic, blood, bone marrow ,tissues (lymph node, tissue biopsies) and fine needle aspirates (FNAs). Specimens contaminated by normal flora or specimens not collected aseptically (not sterile) –gastric lavage, bronchial washing, urine, pus and stool (in case of disseminated TB in HIV infected patients)

METHODS:

Various body fluids submitted to the Department of Microbiology, obtained from patient for Microbiological testing for confirmation of diagnosis of TB were prospectively studied. These included body fluids (CSF, pericardial fluid, pleural fluid, peritoneal fluid, peritoneal dialysis fluid, synovial fluid) Urine and Pus. Immediate processing using standard microbiological methods were done which included digestion-decontamination, Microscopy, Culture and Real Time PCR.

RESULTS :

During this study period 190 extra pulmonary specimens were submitted for diagnosis of *Mycobacterium tuberculosis* infection by PCR, Culture and smear. The distribution of various samples with their Results in EPTB Samples (n=190) was shown in Table 1.

Table 1: Distribution of various samples with their Results in EPTB Samples (n=190).

S. No	Nature of Sample	Total	Smear Positive	Culture Positive	PCR Positive	PCR & Culture Positive	Smear & Culture Positive	All Positives
1	CSF	34	NIL	2	3	1	NIL	……
2	PD FLUID	40	NIL	3	3	2	NIL	……

3	URINE	51	NIL	4	1	1	NIL	……
4	PUS	16	1	2	7	2	1	1
5	ASCITIC FLUID	14	NIL	NIL	1	NIL	NIL	……
6	BONE MARROW	5	NIL	NIL	NIL	NIL	NIL	……
7	PLEURAL FLUID	23	NIL	1	4	1	NIL	……
8	SYNOVIAL FLUID	4	NIL	NIL	1	NIL	NIL	……
9	PERICARDIAL FLUID	3	1	2	1	1	1	……
	Total	190	2	14	21	8	2	1

Out of 190 clinical suspected cases of EPTB, Real Time PCR was detected maximum number 21(77.77%). ZN staining is positive in only 2(7.40%) and culture which is a gold standard method for *Mycobacterium tuberculosis* detected only 14(51.85%). This difference in the sensitivity rate of PCR, LJ media and ZN Staining is statistically significant ($p<0.05$).

DISCUSSION

In our study, a total of 27 out of 190 clinically suspected cases of extra pulmonary tuberculosis were confirmed by the different diagnosis methods. In the present study 2 (7.40%) were positive by ZN staining. Culture is the established gold standard for the diagnosing tuberculosis, though it lacks sensitivity, specificity and speed, especially in the detection of EPTB. In the present study, only 14(51.85%) of the clinical samples from the suspected EPTB cases yielded growth on LJMedium.

Real Time PCR has very high specificities, variable sensitivities and speed. Whenever it was properly executed, PCR has proved to be a very sensitive test in EPTB. In the present study Real time PCR has detected maximum number 21(77.77%) of the EPTB samples. The conventional methods together could detect 51.85% only. All the smear and culture positive samples were also found to be positive by Real Time PCR. But one sample is positive for conventional methods but negative in PCR because it is atypical *Mycobacterium tuberculosis.*

In the present study, PCR proved to be a very effective rapid test, with a significant p value (0.001). The difference in the results was also statistically significant when PCR was compared with the culture. As a single diagnostic test, PCR was the most successful method for the diagnosis of EPTB in my study. Real time PCR was negative in one sample (pus) that was found to be positive by the conventional tests. The negative PCR results may be attributable to the nil or very low number of copies. The kit used for PCR is HELINI Biomolecule the target sequence (Rep 13e12) is highly conserved used for designing.

Maximum numbers of patients suffering from extra pulmonary tuberculosis were in the age group of adults. Out of 27 positives 24 were adult age group. Followed by elders 2 positives and in the age group children's only one positive. Our findings were nearly correlated with siddiqui *et al.,* 2013.[13] In our study, the overall positivity rate of PCR was found to be 77.77%. Negi *et al* and chewla *et al* using the same set of primers reported the overall positivity rate to be 77.2% and 74.1%.[14] In our study, the sensitivity of PCR in detecting LJ Medium positive samples was 100% and in detecting LJ medium negative samples was 48.15%. Our study is comparable to those of negi *et al* and Kesarwani *et al* in PCR positivity in culture positive cases. In our study maximum samples were positive in case of pus 7 out of 27 positives (25.92%), followed by CSF samples were positive for 4 out of 27(14.81%), PD fluid samples were positive for 4 out of 27(14.81%), URINE samples were positive for 4 out of 27(14.81%), Pleural fluid samples were positive for 4 out of 27(14.81%) , pericardial fluid samples were positive is 2 out of 27 positives(7.40%), ascetic fluid and synovial fluid samples were positive only one out of 27 positives (3.70%) and bone marrow could not show any positive with any method in our study.

The diagnosis of EPTB is made difficult by the complexity and risks involved in the collection of specimen for examination. It is challenging for a number of reasons: the lack of adequate sample amounts or volumes; the apportioning of the sample for various diagnostic tests (histology/cytology, biochemical analysis, microbiology and PCR), resulting in non uniform distribution of microorganisms; the pacuibacillary nature of the specimens; the presence of inhibitors that undermine the performance of nucleic acid amplification based techniques; and the lack of an efficient sample processing technique universally applicable on all types of extra pulmonary samples. This leaves clinicians with a longer delay without an affirmative answer. But with the development of newer techniques in molecular biology, this delay in the accurate diagnosis of the disease is minimized.

In a study in Turkey by Ozkutuk evaluating the performance of Cobas Amplicor MTB (CAMTB) test for pulmonary and extra pulmonary tuberculosis specimens, results indicate that the CA-MTB is a rapid test for detection of tuberculosis in pulmonary specimens, but does not perform well enough in extra pulmonary specimen. The poor performance of conventional microbiological techniques in extra pulmonary specimens has stimulated the increased use of PCR tests in the laboratory diagnosis of tuberculosis. [15]

Real –Time PCR is highly sensitive and widely accepted method for the diagnosis of any kind of infectious disease. The primer used in this study is very specific for *Mycobacterium tuberculosis*. The target sequence used in our study is Rep13e12, but it has been also reported in some earlier studies that PCR assay targeting Rep13e12 were more sensitive and more useful compared to other targets like IS6110, 65KDa, 38KDa, and 85B protein. The methodology of PCR for Rep13e12 has been also carried out in different technical setups and has been proven to be simple and reproducible, compared to methodologies for PCR targeting other gene sequences. In this study the accuracy of primers was confirmed by using positive control which crossed the threshold level after certain cycles of amplification. In this study higher percentage of positive results were found when Real –Time PCR analysis was carried out in all specimens except in pus sample.

Though it is reported in earlier studies about the usefulness and sensitivity of IS6110 over AFB microscopy and culture method using conventional PCR method, but Real-Time PCR could be much more useful in terms of rapidity of producing perfect result. The rapidity, high sensitivity and simplicity of PCR targeting Rep13e12 gene sequence can compensate the higher cost of the test compared with less sensitive conventional tests in the diagnosis of extra pulmonary tuberculosis and facilitate the therapeutic decision made clinician.

CONCLUSIONS

India is endemic for the tuberculosis and with introduction of HIV infection the situation has aggravated further. This created a burden on different laboratories hard pressed for the prompt detection and reporting of Mycobacteria. The diagnosis of EPTB in its different clinical presentation remains a true challenge. Conventional diagnostic tests like smear examination for acid fast bacilli culture identification have serious limitation of sensitivity and the long time taken for growth of the bacilli. PCR was found to be the most rapid method for the diagnosis of tuberculosis, being extremely useful in diagnosis of extra pulmonary tuberculosis. Rapid results by PCR allow quick implementation of treatment regimen.

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