ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 07 | July - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DIFFERENTIAL SENSITIVITY OF PCR TARGETS TO DETECT M. TUBERCULOSIS IN TUBERCULOUS PLEURAL EFFUSION SPECIMENS

Biochemistry

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ABSTRACT

Tuberculous pleurisy is the second most popular form of the Extra pulmonary Tuberculosis disease in India. It is fairly tricky to identify these patients due to the variable symptoms presented by them and lack of prescribed guidelines for its diagnosis. Documentation of *M tuberculosis* in the pleural effusion sample is the first step in making a successful diagnosis. The present study is based on 59 Physician diagnosed Tuberculous pleurisy patients from Amritsar, North India. Using PCR, IS 6110 elements could be detected in 95% of tuberculous pleural effusion samples and only 78% specimens gave a positive result with 38kDa gene. The conventional tests were of limited use as microscopy and Culture could detect *M. tuberculosis* in only 3 and 23% of pleural effusion samples, respectively. The high sensitivity of IS6110 elements-based PCR test revalidates that *M. tuberculosis* isolates could be reliable detected in tuberculous pleural effusion samples and prevalent *M tuberculosis* isolates from Punjab, North India differ from those in South India.

KEYWORDS

Tuberculous Pleurisy, EPTB, *M tuberculosis*, PCR, IS6110, 38kDa,

INTRODUCTION

Tuberculosis (TB) is a bacteria infectious disease, caused by *Mycobacterium tuberculosis* complex organisms (MTB). TB primarily affects the lungs (pulmonary tuberculosis - PTB) but can affect other organs of the body (extra pulmonary tuberculosis-EPTB). India alone accounts for ~27% of the global TB burden and holds the key to the global eradication of this disease [1]. Tremendous progress has been made in diagnosing and treating PTB patients, but much is still desired for diagnosing EPTB. In India, Tuberculous pleurisy is one of the most common manifestations of extra-pulmonary TB after lymphadenitis and is a diagnostic challenge.[2] In this clinical condition, *M tuberculosis* infects the pleural space resulting in the accumulation of fluids and inflammatory cells. The accumulated fluid is referred to as Tuberculous pleural effusion (TPE). For the diagnosis of TPE, no formal guidelines are available. The gold standard test for diagnosing tuberculous pleurisy is pleural fluid culture, however, it is universally accepted that this test is of limited use due to its low sensitivity.[3] Pleural biopsy is another test used for diagnosing EPTB, but that necessitates invasive procedures and can be performed in specialized centres only.

The polymerase chain reaction (PCR) technique has emerged as a rapid and readily available tool for the detection of *M. tuberculosis* in clinical samples.IS 6110 is one of the most widely studied targets.[3][4][5]. This insertion element is 1.35 Kb long, a member of IS-3 family, known to be present in multiple copies. It is widely used in epidemiological studies, especially in identifying tuberculosis outbreaks, due to its high degree of stability in the mycobacterial chromosome.[6][7] However, it's use in Indian isolates has been challenged due to the lack of these elements in *M tuberculosis* isolates from South India and a need for alternative target(s) for *M. tuberculosis* detection is warranted[8]

Keeping these observations in view, the present study was undertaken to evaluate the sensitivity of two *M. tuberculosis* targets for the diagnosis of tuberculous pleurisy in TB patients of Punjab in North India.

MATERIAL AND METHODS:

Clinical Specimens and Culture:

Fifty-Nine exudative Pleural effusion specimens, with protein more than 3g% and ADA value of more than 40 IU/L, were collected randomly from physician diagnosed tuberculosis patients (24 females and 35 males) visiting Sri Guru Ram Das University of Health Sciences, Amritsar. The study was conducted during 2018-2023 and requisite ethical clearance was accorded by the institution. The specimens were decontaminated and stained for the acid-fast bacilli according to standard Mycobacteriological procedures. A portion of the pleural fluid specimen was inoculated on LJ medium and observed regularly, upto 10 weeks. The positive culture was reconfirmed following Z-N staining and Niacin test.[9]

DNA isolation:

Mycobacterial DNA was isolated from clinical sample and the known Niacin positive cultures of *M. tuberculosis* using modified Freezing and Thawing protocol[10].

PCR (Amplification of mycobacterial DNA)

The genomic DNA isolated by the above protocols was amplified for two *M. tuberculosis* targets, gene for Pab antigen (38kDa) as described by Dwivedi and Sehajpal [11] and IS 6110 insertion elements following Eisenach *et al* [12] protocol. The amplified PCR products were analyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining.

RESULTS:

The age of studied Tuberculous pleurisy patients (N=59) ranged between 12 and 76 years, with an average of 3715.6 years. Fifty nine percent of participants were males (35/59) and the male to female ratio was 1.45 :1.

A total of 59 random tuberculous pleural effusion samples, one from each patient, were subjected to AFB staining and LJ culture.

Table 1. Microbiological analysis of Pleural fluid samples

No of samples tested	Microscopy by ZN Method		Culture on LJ medium	
	AFB+ve	AFB-ve	Culture+ve	Culture-ve
59	1 (3.75%)	58 (96.25%)	14 (23.73%)	45 (76.27%)

Table 1 gives the results of microscopy and culture tests. Only 1/59 pleural effusion sample was found to be positive for AFB staining and 14/59 scored positive on the LJ culture. All the culture were found to be AFB and Niacin positive. Table 2 gives the results of PCR test performed on DNA isolated from 59 pleural effusion samples.

Table 2. Efficiency of 38-kDa and IS 6110 genes targets for the detection of *M. tuberculosis* in the clinical Tuberculous pleural effusion samples.

No of samples tested	PCR for the 38kDa gene PCR +ve (%)	PCR –ve (%)	PCR for the IS 6110 gene PCR +ve (%)	PCR –ve (%)
59	46 (77.96%)	13 (22.03%)	56 (94.92%)	3 (5.08%)

Chi square = 4.112; p = 0.042

The PCR test was targeted for 38kDa gene and IS 6110 insertion elements present in the *M. tuberculosis* genome. It is evident from the table that 77.96 % and 94.92% samples were found to be positive for 38kDa and IS 6110 genes, respectively. The sensitivity of the two tests was compared and the differences were found to be statistically significant (p=0.04). It is pertinent to add that all culture positive pleural effusion samples gave positive result with both the selected targets.

DISCUSSION

Diagnosing EPTB is a daunting challenge. To address this emerging conundrum, the Government of India constituted a committee to prepare a guideline to meet this challenge. A salient recommendation of the committee was to make Nucleic acid amplification methods as part of the routine diagnostic tests, keeping in view that standard microbiological tests are of limited use in diagnosing EPTB[13].

In this study only 3 and 23% cases could be identified using microscopy and microbial culture, respectively (Table1). These findings are in line with earlier observations [14] [15] [16].

PCR emerged as a sensitive technique in the detection of *M. tuberculosis* from clinical samples and IS 6110 is the most widely used target in this test [3][4][16]. In this study, IS 6110 emerged a more sensitive target compared to the 38kDa gene, as the former could detect MTB genome in 95% of pleural effusion samples compared to 78% with the latter one (Table 2). IS 6110 being multi copy element offers multiple targets per bacterium which accounts for its higher sensitivity in tuberculous pleural effusion samples. It is pertinent to add that the selection of TPE specimens in this study were more stringent, i.e samples having > more 3g% protein and ADA > 40 IU/L, and probably contributed in a high PCR sensitivity for MTB in pleural effusion samples. The high incidence of clinical isolates lacking the IS 6110 elements in Indian populations raises a question mark regarding the utility of this marker as a diagnostic tool in the detection of *M. tuberculosis* [6] [7] [8]. Radhakrishnan et al [8] reported that 50/80 *M. tuberculosis* isolates from Kerala, South India could not be identified using IS 6110 elements and therefore suggested a need for alternative target(s) for *M tuberculosis* detection.

On the contrary, this study documented that IS6110 is a better target as compared to the single copy elements like 38kDA gene for the detection of *M. tuberculosis* in North Indian TB population (Table 2). This also validates our earlier finding that *M tuberculosis* clinical isolates are predominantly modern in Punjab, North India and are different from those prevalent in South India[17].

It is safe to conclude that PCR is a rapid, reliable and sensitive technique for the detection of *M. tuberculosis* in pleural effusion samples and IS 6110-multi copy elements is a far better target compared to single copy genetic element like 38kDA gene, in North Indian Tuberculous pleurisy patients. More studies validating the use of PCR in diagnosing paucibacillary form of TB should be encouraged to generate a more comprehensive data on this important aspect of public health

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