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Research Article

Dual Targets in PCR and its Usage for the Molecular Characterization of *Mycobacterium Tuberculosis*

Rachana Panwar[2], Udita Bharti[2], Priyanka Bhatt[2], Ankit Rana[2], Sheetal Verma[3], Neelam Negi[2], Narotam Sharma[1]

[1]Central Molecular Research Laboratory, Biochemistry Department, SGRRIM&HS, Patel Nagar, Dehradun, Uttarakhand- India
[2]DBS (PG) College, Dehradun, Uttarakhand-India
[3]GLA University, Mathura, U.P. India

***Corresponding author**
Dr. Narotam Sharma
Email: sharmanarotam5@gmail.com

Abstract: Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis.* Diagnostics methods includes Serological, Radiological and Microbiological investigations. The current study was done on the dual targets i.e. *IS6110* and *mpb-64* for the characterization of tuberculosis in 25 cases with the usage of Nested PCR, and Conventional PCR. 12 patients were negative and 13 were positive for Tuberculosis PCR. Further it was observed that out of 13 positive patients all gave positive target amplification for *IS6110* gene whereas only 5 of them were observed positive for *mpb64*. Thus *IS6110* is a better molecular marker for the PCR reaction when used in routine diagnosis.
Keywords: Polymerase chain reaction, Nested PCR, Mycobacterium tuberculosis, uracil-N-glycosylase.

INTRODUCTION

Mycobacterium tuberculosis (MTB) is a pathogenic bacterial species in the family Mycobacteriaceae and the causative agent of most cases of tuberculosis (TB). *M. tuberculosis* is genetically diverse, which results in significant phenotypic differences between clinical isolates [1]. Different strains of *M. tuberculosis* are associated with different geographic regions. However, phenotypic studies suggest that strain variation never has implications for the development of new diagnostics and vaccines. Microevolutionary variation does affect the relative fitness and transmission dynamics of antibiotic-resistant strains [2-4]. Diagnosis of tuberculosis is made thorough clinical history, including symptoms, and performing a physical examination [5]. Tests include special blood tests and a tuberculin skin test, which can detect if a person has been infected with the *Mycobacterium tuberculosis* bacterium or has had a vaccination for tuberculosis [6]. Lesions in the lungs that are due to tuberculosis may also be seen on a chest X-ray. These tests cannot detect if the infection has lead to active tuberculosis. Diagnosis of tuberculosis can be delayed or overlooked because there may be no symptoms. Molecular approaches targeting different markers is of utmost significance [7,8]. *IS6110* was first described by Thierry et al. *IS6110* gene - *IS6110* is an insertion sequence element found exclusively within the members of the Mycobacterium tuberculosis complex (MTBC), and because of this exclusivity, it has become an important diagnostic tool in the identification of MTBC species [9]. *IS6110* is 1,361 bp long and contains 28-bp, imperfect inverted repeats at its extremities with three mismatches and 3-bp direct repeats that probably result from repetition of the target sequence among the various mycobacterial species [10]. The restriction of *IS6110* to the MTBC is hypothesized to arise from the inability of these bacteria to exchange DNA [11]. The presence of *IS6110* indicates that lateral gene transfer has occurred among mycobacterial species, suggesting that the mycobacterial gene pool is larger than previously suspected [12,13]. Moreover, the element's presence in multiple copies and at differing locations in the genome, has provided an excellent method by which strains can be genotyped; because of these characteristics, *IS6110* has been used extensively for epidemiological studies different clinical samples [14, 15]. *IS6110* is a novel Mycobacterial insertion element formed the basis of a reproducible genotyping technique. The current study was done on the *IS6110* and *mpb-64* as targets for the diagnosis of *Mycobacterium tuberculosis* complex in clinical specimens

MATERIALS AND METHODS

A total of 25 clinical specimens were collected for the proposed study from the different Departments of SMI Hospital, Dehradun, Uttarakhand. All the

specimens were subjected for amplification of the dual targets, *mpb-64* and *IS6110* by conventional and nested PCR respectively. All the CSF specimens were subjected parallel for MTB complex detection by nested PCR using uracil-N-glycosylase (UNG) enzyme in pre-mix targeting *IS6110* by conventional PCR using *mpb-64* gene. All the protocols were subjected with controlled parameters utilizing nuclease free water as negative control where after every three specimens a negative control was processed to check any sort of contamination. Nested PCR was performed utilizing manufacturer protocol (Bangalore genei). In case of nested PCR, an amplification product of size 123 bp was indicative of infection with *Mycobacterium tuberculosis* complex where as the amplification product of internal control DNA was 340 bp. In case of conventional PCR only 123 base pair product indicates Mycobacterial infection as depicted in figure.

RESULTS

The 2 different variants of PCR 1.Nested PCR, and 2.Coventional PCR were performed for the molecular characterization of *Mycobacterium tuberculosis*. 25 samples of suspected and symptomatic patients were screened out to detect the target gene for tuberculosis i.e *mpb64* and *IS6110*. Out of the overall symptomatic patients twelve patients were negative and thirteen were positive for Tuberculosis. And from those 13 positive patients all gave positive target amplification for *IS6110* gene whereas only 5 of them were observed positive for *mpb64*.

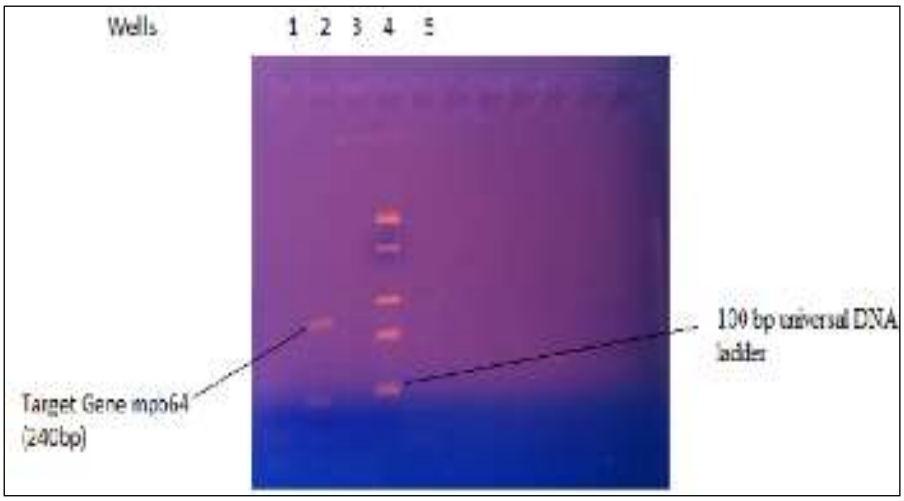

Fig-1: Gel picture showing the positive result of MTB by conventional PCR

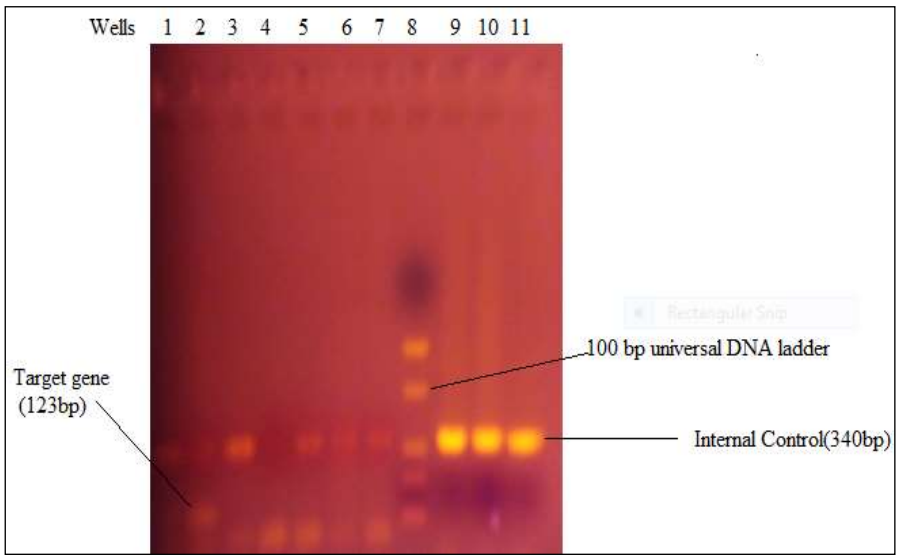

Fig-2:Gel pic showing Positive result for MTB in well 2, validated with Internal Control

DISCUSSION AND CONCLUSION

During the past 30 years molecular techniques have been under development, however these have had a rapid and tremendous progress in recent year [16, 17]. Among molecular techniques, PCR and its different variations are highlighted as the most commonly used in laboratories and research institutes [18]. Thus, these have contributed to identification and characterization of several organisms and understanding of physiopathology of diverse diseases in human, animal and plant. Also these have provided clues for future research directions in specific topics with impact in

public health such as genetics and biochemistry of antimicrobial resistance [19]. The following describes some applications of PCR and its variants in studies in human medicine, forensic sciences, and agricultural science and environment. One month is not enough for performing exact molecular characterization of any microorganism. In the present study two genes *IS6110* and *mpb64* have been compared with two variants of PCR (Nested and Conventional) in the diagnosis of *Mycobacterium tuberculosis.* The results of the present study showed that the system targeting Insertion Sequence *IS6110* appears to be a better target of amplification as compared to *mpb64*.Several factors play an important role in the efficacy of a PCR protocol . The molecular mass and the size of the target gene matters a lot. Larger the size more are the chances of degradation and thus lower the efficacy of product. This suggests the need to chose the correct primers, such as targeting repetitive elements and those amplifying relatively shorter DNA sequences, which are less prone to fragmentation. In the present study the positive signals obtained for symptomatic patients where higher with *IS6110* (13/13 i.e 100%) and lower with *mpb64* (5/13 i.e 38.46%). It has been studied early that the Indian subcontinent has *Mycobacterium* with high copy number of *IS6110.*

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