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Original Article

Molecular Characterization of *Mycobacterium tuberculosis* using *IS6110* Gene with Nested PCR-Clinical Relevance for the Disease Characterization

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ABSTRACT

Mycobacterium tuberculosis (MTB) is a pathogenic bacteria species in the genus Mycobacterium and the causative agent of most cases of tuberculosis. Work was conducted using a faster and advanced method of Nucleic Acid Amplification Technology to characterize *Mycobacterium tuberculosis* using *IS6110* gene. 50 Clinical samples were collected, DNA was isolated using silica column method, amplification of DNA in Nested PCR machine, post amplification using Agarose Gel Electrophoresis and result interpretation in E-gel Imager trans-illuminator. Of 50 clinical samples processed, 10(20%) were positive and 40(80%) were negative for *IS6110* gene. In age-wise distribution, 21-60(years) had 32(64%) clinical samples with highest positivity rate of 9(28.1%) compared to other age group distributions and 23(78.9%) were negative. In gender wise distribution, 34(68%) were males with 9(26.5%) positive and 25(73.5%) negative. The female clinical samples were 16(32%), of which 1(6.3%) was positive and 15(93.7%) were negative. These results were in accordance to other researches carried out with *IS6110* gene as target. Amplification technologies offer the potential for the diagnosis of tuberculosis in a few hours with a high degree of sensitivity and specificity. To prevent the spread of the disease, early diagnosis using molecular assay especially with *IS6110* or *mpb64* as target gene is more efficient due to the long incubation period of tuberculosis which does not favor culturing process and prompt treatment of cases being the best option.

Keywords: *Mycobacterium tuberculosis*, Nested Polymerase Chain Reaction, *IS6110* gene.

1. INTRODUCTION

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis, infects one third of humans and causes 3 million deaths annually [1, 2]. It is the etiologic agent of tuberculosis in humans, the only reservoir for the bacterium.

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Tuberculosis (TB), one of the oldest recorded human afflictions, is still one of the biggest killers among the infectious diseases, despite the worldwide use of a live attenuated vaccine and several antibiotics [3, 4]. TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages [5, 6]. The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe [7, 8]. Tuberculosis of the lungs may also occur via infection from the blood stream. This is known as a Simon focus and is typically found in the top of the lung [5, 9]. This hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and the bones [10]

2. MATERIALS AND METHODOLOGY

Sample Collection: 50 clinical samples were collected from the inwards and outwards patients in various Departments of Shri Mahant Indiresh Hospital, Patel Nagar, Dehradun, Uttarakhand, India. **DNA Isolation**: Genomic DNA isolation was done using the silica column method. **Amplification**: The master mix composition for first amplification was TB PCR mix-5µl, $MgCl_2$- 2µl, Taq DNA Polymerase-0.25µl, forward primer (5` - CCT GCG AGC GTA GGC GTC GG - 3`) - 0.2µl, reverse primer (5` - CTC GTC CAG CGC CGC TTC GG - 3`) - 0.2µl, dNTPs- 0.2µl, Nuclease Free Water-10.35µl, DNA Template-10µl. Cycling Condition for *IS6110* in a Conventional Thermal Cycler was done as follows: **Segment 1**: Initial denaturation at 94°C for 10minutes, **Segment 2: Step 1**: Denaturation at 94°C for 00:30seconds. **Step 2**: Annealing of 35cycles at 65°C for 1minute, **Step 3**: Extension at 72°C for 1minute, **Segment 3**: Final Extensions at 72°C for 7minutes and storage at 4°C for 99minutes. **Post Amplification:** Post amplification was done using 1.6% Agarose Gel Electrophoresis with 125V of electricity for 12minutes and results were viewed using E-gel imager trans-illuminator.

3.RESULTS

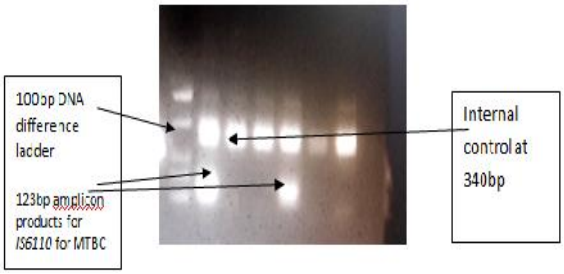

Fig 1: E-Gel imager and results interpretation Out of the 50 clinical samples obtained and processed, findings includes, as tabulated in table below:

Table 1: Different clinical samples processed with respect to positivity and negativity

S/N	Samples	Number of Cases	Positive (%)	Negative (%)
1	Cerebrospinal Fluid(CSF)	27(54%)	5(54%)	22(46%)
2	Pleural Fluid(PF)	3(6%)	0(0%)	3(100%)
3	Bronchoaveolar Lavage(BAL)	7(14%)	4(42.2%)	3(57.1%)
4	Synovial Fluid(SF)	2(4%)	0(0%)	2(100%)
5	Ascetic Fluid(AF)	3(6%)	1(33.3%)	2(66.7%)
6	Blood	1(2%)	0(0%)	1(100%)
7	Pus	3(6%)	1(33.3%)	2(66.7%)
8	Urine	1(2%)	0(0%)	1(100%)
9	Tissue	3(6%)	1(33.3%)	21(66.7%)
	Total	50(100%)	12(24%)	38(76%)

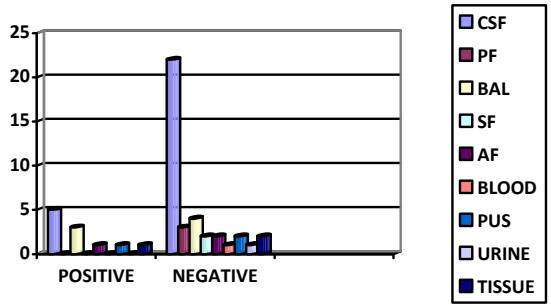

Fig 2: Positive and negative cases of MTB with respect to samples type

As shown in table 1 and figure 2 above;

Cerebrospinal Fluid (CSF): Out of 50 of the clinical samples, CSF was 27(54%) of the samples of which 5(18.5%) were positive to *IS6110* and 22(81.5%) were negative. **Pleural Fluid (PF):** Pleural fluid constituted 3(6%) of the clinical samples of which 0(0%) was positive and 3(100%) negative. **Bronchoaveolar Lavage (BAL): 7**(14%) of the samples was BAL, out of which 3(42.9%) were positive and 4(57.1%) were negative to *IS6110*. **Synovial Fluid (SF):** The joint fluids or SF were 2(4%) of the samples. There was no positive outcome with *IS6100* as 0(0%) was positive and 2(100%) were negative. **Ascetic Fluid (AF):** AF represented 3(6%) of the samples. 1 (33.3%) showed a positive result while 2(66.7%) showed negative result. **Blood:** Blood made up just 1 (2%) of the samples and was 0(0%) positive and 1(100%) negative. **Pus:** 3(6%) samples were from pus of which 1(33.3%) was positive and 2(66.7%) negative to the *IS6110* gene. **Urine:** 1 (2%) sample came from urine and 0 (0%) positive results were obtained while 1(100%) negative was seen. **Tissue:** 3(6%) samples were got from different unspecified tissues in the body. 1(33.3%) of the tissues showed a positive result while 2 (66.7%) were positive.

Age wise positivity and negativity rates for *Mycobacterium tuberculosis*

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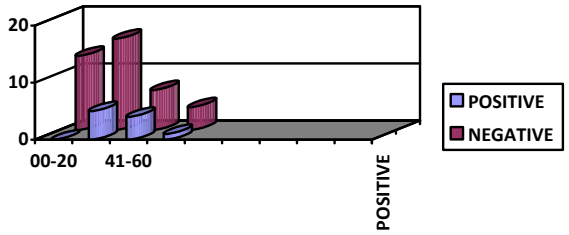

Fig 3: Positive and negative cases of MTB infection with respect to age groups

The samples were also distributed according to ages of 00-20 class intervals and results obtained were as shown in figure 3; 00-20(years) age distribution had a total of 13(26%) of the samples out of which 0(0%) was positive and 13(100%) negative for *IS6110* gene. Group 21-40(years) made up of 21 (42%) of the samples with 5(23.8%) positive results and 16(76.2%) negative results. Age distribution 41-60(years), had 11(22%) samples, 4(36.4%) showed positive for *IS6100* while 7(63.6%) showed negative results. In group above 61years, 5(10%) samples were processed and 1(20%) positive results were obtained while 4(80%) were negative.

Gender wise positivity and negativity rates for Mycobacterium tuberculosis

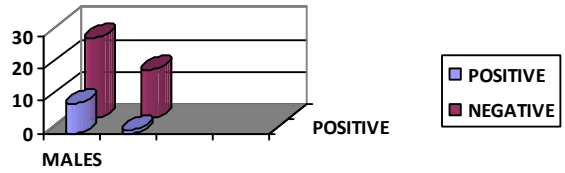

Fig 4: Showing male and female positive and negative samples

As shown in figure 4, of the 50 clinical samples processed, 34(68%) were males. The results showed that 9(26.5%) of the males were positive while 25(73.5%) were negative. In the female category, out of the 50 samples, 16(32%) were females which 1(6.3%) was positive and 15(93.7%) negative.

4. DISCUSSION AND CONCLUSION

Tuberculosis is a contagious bacterial infection that involves organs other than the lungs [11]. Tuberculosis is caused by the bacteria *Mycobacterium tuberculosis* (M.tuberculosis) [12]. Tuberculosis affects people with weak immune system, diabetes, HIV, or malnourished people, very young or elderly, those undergoing prolonged treatment with Chemotherapy or cortisone [14]. Amplification technologies offer the potential for the diagnosis of tuberculosis in a few hours with a high degree of sensitivity and specificity [15]. *IS6110* is an insertion element that is found exclusively within the MTBC; the assumption has been that this restriction is a result of the lack of genetic exchange with other Mycobacterial Species [3, 16].

The results of this work have shown that MTB is not peculiar to a particular race, tribe, age, gender and geographical locations. As found in other researches, TB is an infectious and deadly disease caused by *Mycobacterium tuberculosis* and has a long incubation period [11]. In India, about 70% of the cases of *Mycobacterium tuberculosis* occur among the age group of 15-54 years [10, 17], which also corresponded to the findings in this work whereby age groups 21-60 years had more positive results with *IS6110* gene than other groups. Out of 9.4 million cases occurring worldwide per year, nearly 2 million cases in India, that is, 1/5th of the global burden, and 2/3rd of South East Asian Region [18]. This was also indicated in this work as 10(20%) samples were positive and 40(80%) negative. People with compromised immune systems are most at risk of developing active tuberculosis [19]

HIV suppresses the immune system, making it harder for the body to control TB bacteria. People who are infected with both HIV and TB are around 20-30% more likely to develop active TB than those who do not have HIV [20]. Some people may develop tuberculosis in an organ other than their lung [21] as also found in this work in which clinical samples such as Cerebrospinal Fluid (CSF), Pus and Endometrium indicated positively to *Mycobacterium tuberculosis*. TB is an infectious disease caused by bacteria *Mycobacterium tuberculosis*. To prevent the spread of the disease, early diagnosis using molecular assay especially with *IS6110* or *mpb64* as target gene due to long incubation period of TB which does not favor culturing process and prompt treatment of cases is the best option.

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