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Research Article

COMPARATIVE STUDY OF MICROSCOPY AND PCR IN DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* IN A TERTIARY CARE CENTRE

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Abstract- Background & objectives: Tuberculosis (TB) continues to be the second the major cause of morbidity and mortality in India as well as worldwide. Early diagnosis is required to prevent transmission of TB and also to reach the WHO goal to end TB by 2035. TB is diagnosed in laboratories by smear microscopy and culture which either lacks sensitivity or delay in reporting. Hence, RT-PCR was compared with sputum microscopy to find an appropriate method to diagnose TB at the earliest. Methods: Forty-four sputum samples were collected from presumptive TB cases, sputum microscopy was done by Ziehl-Neelsen stain, Auramine O stain microscopy and RT-PCR was done by using primer IS6110 of *Mycobacterium tuberculosis*. Results: Of the forty-four sputum samples, ZN staining showed 48% positivity while Fluorescent stain showed 55% positivity in smear and PCR showed 71% positivity in the sputum samples. Interpretation & conclusions: PCR is a better method than staining techniques for detection of *Mycobacterium tuberculosis*. Fluorescent microscopy is more sensitive when compared to ZN stain but requires an expensive microscope.

Keywords- *Auramine O stain, IS6110, Microscopy, Mycobacterium tuberculosis, Polymerase Chain Reaction, Ziehl-Neelsen stain*

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Introduction

Mycobacterium tuberculosis is a gram-positive bacillus which causes tuberculosis affecting the lungs primarily and other organs like intestines, meninges, bones, lymph nodes, skin and other tissues. Tuberculosis is one of the top ten leading causes of death by a single agent with estimated 1.3 million deaths worldwide. Around 10.0 million people have developed TB disease in 2017of which there were 5.8 million men, 3.2 million women and 1.0 million children [1]. Therefore, early diagnosis and treatment is very essential in prevention of the disease and to get closer to the goal of Revised National Tuberculosis Control Program (RNTCP) to End tuberculosis by 2025 [2]. Conventional diagnostic methods for tuberculosis include clinical history, sputum microscopy, skin test and culture. Staining techniques and skin test are the most commonly used methods in resource poor countries [3]. Ziehl-Neelsen staining is rapid and cheaper method when compared to the fluorescent stain. But Fluorescent microscopy is better than ZN stain in the detection of tubercle bacilli [4]. Both these methods lack sensitivity in case of low microbial load as seen in Human immunodeficiency virus infection [5]. The gold standard for diagnosis of tuberculosis is culture. But sensitivity and specificity of culture is very low as shown by some studies [6]. Polymerase chain reaction (PCR) is a molecular technology which amplifies a single copy or few copies of deoxyribonucleic acid (DNA) into thousands or millions of copies which will be identified by TB specific gene like IS6110. PCR is costlier when compared to other methods butis more sensitive than staining methods. PCR gives quicker results when compared to culture [7, 8]. A rampant disease like tuberculosis requires a test which is rapid, sensitive and specific to the disease [6]. Hence the objective of this study was to compare the performance and effectiveness of microscopy and RT-PCR in detection of tuberculosis.

Material and Methods

This study was conducted in the Department of Microbiology in Rajarajeswari Medical College & Hospital, Bengaluru from March to May 2017. Institutional ethical committee approval has been obtained for this study. Total of 44 sputum samples were tested from patients attending both out-patient and in-patient departments. Patients of all ages and both genders were included in the study. Sputum samples were collected in a sterile, leak proof, wide mouthed containers with proper labeling. Samples were subjected to Ziehl-Neelsen (ZN), Auramine O stain and real time polymerase chain reaction (RT-PCR). ZN staining was done on the sodium hydroxide (NaOH) processed sputum samples and examined for presence of acid-fast bacilli. Bacilli were seen as pink colored rods against a blue background. Fluorescent staining was done using Auramine O and examined under 40X. The bacilli were seen as bright rods against a dark background. Smears were graded according to RNTCP guidelines [9].

RT-PCR

Sputum samples were decontaminated and extraction of DNA was done as per manufacturer's protocol (MACHEREY-NAGEL GmbH & Co. KG, Duren, Germany). Internal positive control was added while extracting the DNA for validation of RT-PCR amplification. The obtained DNA was quantified by nanodrop 2000C (Thermoscientific). After quantification, the DNA was further processed by RT-PCR amplification using Amplisens MTC-FRT PCR kit, Moscow, Russia. The amplification was done in step one RT-PCR (Applied BiosystemsStepOne Instrument) by specific primers IS6110. The samples were run along with Positive and negative controls. The data was analyzed using descriptive data analyses in SPSS. Sensitivity and specificity were compared between stains and PCR. The Kappa coefficient (κ) was used to verify the concordance between the PCR, ZN and Fluorescent stains.

Results

Forty-four sputum samples from presumptive cases were studied. [Table-1] shows positivity by different staining methods and PCR for IS6110.

ZN staining showed 48% positivity while Fluorescent stain showed 55% positivity in smear and PCR showed 71% positivity in sputum sample. [Table-2] shows results of staining techniques and PCR on sputum samples. PCR showed a positivity of 77.5%. Higher positivity was seen by PCR. [Table-3] shows the kappa coefficients obtained from the comparison of PCR, ZN and Fluorescent stains. [Fig-1] shows the amplification plot of first run which shows positive samples as the curve and negative samples as the flat line.

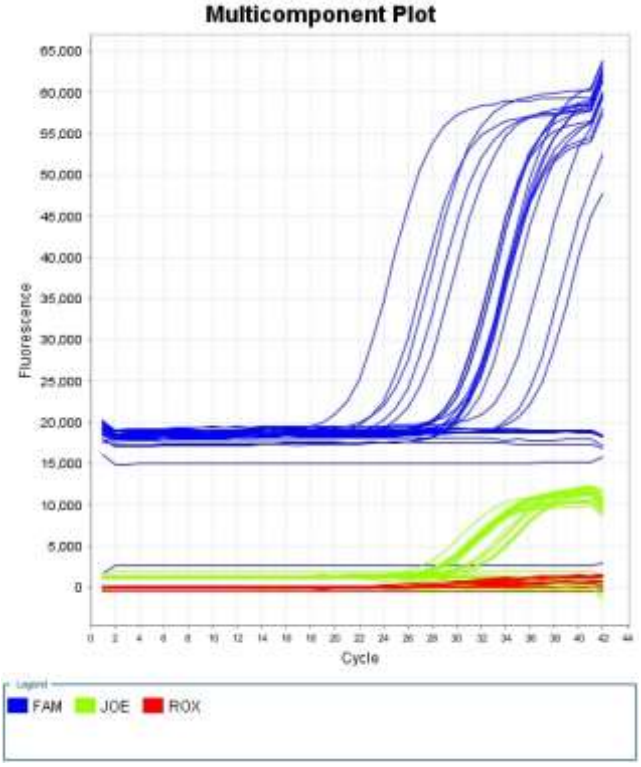

Fig-1 Multicomponent plot of PCR

Table-1 *Number of sputum samples positive for AFB in different staining techniques*

Staining technique	ZN stain	Fluorescent stain	PCR
No of positive samples	21	24	31
No of negative samples	23	20	13
Total	44	44	44

Table-2 *Results of Staining techniques and PCR on sputum sample*

Test	PCR		Sensitivity (%)	Specificity (%)
	Positive	Negative		
ZN stain positive	21	0	67	100
ZN stain negative	10	13		
Fluorescent stain positive	22	2	70	84
Fluorescent stain negative	9	11		
Total	31	13		

Table-3 *Kappa coefficients obtained from the comparison of PCR, ZN and Fluorescent stains*

Diagnostic tests	Kappa coefficient
Ziehl-Neelsen stain vs. PCR	0.55 Moderate correlation
Fluorescent stain vs. PCR	-0.54 Less than chance agreement
Ziehl-Neelsen stain vs. Fluorescent stain	0.86 Strong correlation

Discussion

In India, sputum smear microscopy is still the most commonly used technique for diagnosis of pulmonary TB as it is simple, rapid and inexpensive inspite of low sensitivity [10]. Cultures take several weeks to get results. In this study, fluorescent staining showed 66% positive as compared to ZN staining which showed 47% positivity. This correlates with the study done by Kocagoz *et al*. [11], Saroj *et al*. [12] and Pooja S *et al*. [13] RT-PCR detected a higher number of confirmed TB cases in comparison with ZiehlNeelsen or fluorescent staining methods. This correlates with the studies of Sethi *et al*. [14], Nimesh *et al*. [15] and Pinhata *et al*. [16] Three samples that were negative in ZN stain but was found positive in fluorescent staining. IS6110 is specific to *Mycobacterium tuberculosis* but false positivity is also seen in some cases like *Mycobacterium kansasii* or false negative in some cases like *TB bacilli* which lack IS6110 as shown by Narayanan *et al*. [17] and Radhakrishnan *et al*. [18] will be seen. Approximately 37.5% of the smear negative samples were positive by RT-PCR. This correlates with the study of Nimesh *et al*. RT-PCR showed high specificity, as found by Broccolo *et al*. [19] and Armand *et al*. [20]. Therefore, this study shows that PCR is better than staining techniques but false positive cannot be ruled out.

Conclusion

PCR method is superior to staining techniques for detection of *Mycobacterium tuberculosis*. Fluorescent microscopy is more sensitive when compared to ZN stain but requires an expensive microscope. PCR and fluorescent method require a qualified and personnel with expertise for optimal results.

Application of research: Polymerase chain reaction is more sensitive than compared to other methods of diagnosis for tuberculosis. PCR should be adopted for screening the disease and confirm it by the gold standard method *i.e*., culture.

Research Category: Medical Microbiology

Abbreviations: PCR-Polymerase chain reaction, ZN-Ziehl-Neelsen, TB-Tuberculosis, RT-PCR-Real time polymerase chain reaction, AFB-Acid fast bacilli, RNTCP-Revised National Tuberculosis Control Program, DNA-Deoxyribonucleic acid.

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Study area / Sample Collection: RNTCP Division, TBCD Department, Department of Microbiology, Rajarajeswari Medical College & Hospital.

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Ethical approval: Ethical approval taken from Rajarajeswari Medical College & Hospital, Kumbalgodu, Mysuru Road, Bengaluru, 560074, Karnataka, India.
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