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Molecular Detection of Mycobacterium Tuberculosis Complex from Sputum of Clinically Suspected Tuberculosis Cases

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ABSTRACT

Background: *Tuberculosis (TB) is a communicable disease and it ranks second of all infectious agents due to co-infection with HIV . The causative agent of tuberculosis is a group of mycobacteria known as Mycobacterium tuberculosis complex. Mycobacterium tuberculosis complex consist of M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti. In PCR study, Most commonly sensitivity is higher in smear positive samples (95-100%) rather than smear negative specimens (46-63%).* ***OBJECTIVE****: To detection of Mycobacterium tuberculosis complex by Line Probe Assay.* ***Methods:*** *The study was done from non-interventional approaching study of 50 suspected patients of tuberculosis had visited the TB chest/ DOTS centre. Sputum sample collected early morning in a wide- mouth container from the patients having history of cough more than 2 weeks. Methodology used Z-N staining and for detection of MTB complex was done using MTBDR plus assay, multiplex PCR DNA strip assay (Hain Lifescience, Nehren, Gernamy) which is commercially available. M. tuberculosis secreted an important protein is MPT64, a 24-kDa protein. The major culture filtrate protein (24-kDa) is MPT64 encoded by the RD2 region genes and has been exposed to be an exact antigen that differentiates the M. tuberculosis complex from the mycobacteria other than tuberculosis (MOTT) Species.* ***Results:*** *In 50 samples, out of which 10 (20%) were smear positive & 40(80%) were smear negative. Out of 10 smear positive, 9 (90%) were MTB (Mycobacterium tuberculosis) & 01 (10%) was NTM (Non-tuberculous Mycobacteria) by PCR method. Out of 40 smear negative, 30 (75%) were positive by PCR method. Out of 30, 28(93%) were MTB & 02(7%) were NTM. Rests of the 10(25%) samples were found negative for M. tuberculosis complex.* ***Conclusions:*** *This study proved that PCR is a specific and sensitive method in comparison of sputum microscopy after staining with Z-N technique and it helpful the clinicians ability to diagnose and treat the patients on time. This will ensure early treat to patients and prevent further transmission of disease.*

Key words: *Mycobacterium tuberculosis, communicable diseases, DOTS*

INTRODUCTION

Tuberculosis (TB) is a specific disease which is a leading cause of mortality and morbidity due to bacterial infections in the world and ranks second of all infectious agents due to co-infection with HIV. The causative agent of tuberculosis is a group of mycobacteria known as *Mycobacterium tuberculosis* complex. *Mycobacterium tuberculosis* complex consist of *M. tuberculosis, M. bovis, M. africanum, M.microti, M. canetti.*[1] Diagnostic methods used for the presence of tubercle bacilli on smear positivity in sputum specimens, LJ culture media and X-ray. All above methods have some drawbacks for the diagnosis of tuberculosis. Rapid diagnostic newer methods include nucleic acid amplification method, skin patch test, immune-based assay and culture systems. Line probe assay is a rapid and most sensitive/specific test for the analysis of Drug-resistant strains.[1]

- ZN staining and culture both are at rest main backbone for diagnosis of Mycobacterium tuberculosis. Now a days,

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methods including molecular detection of tuberculosis and this tests have been focused on:-

(i) Detection of nucleic acids DNA by using of amplification technique such as Polymerase chain reaction (PCR) method.

(ii) Detection of gene mutation with different drug resistance strains by sequencing or Nucleic acid hybridization.

Polymerase chain reaction (PCR) is a rapid diagnostic test which is used for tuberculosis that has been evaluated in a large number of studies. In this method, the level of detection is increased by amplification of DNA segments. In all mycobacterial species 64-kDa antigen is present and for the M. tuberculosis complex MPB64 antigen is present for the amplification of DNA sequence coding that has been reported.

Sensitivity of microscopy is 60-70% in culture positive sputum samples and sensitivity of PCR is 90-100% in smear positive, culture positive and 60-70% in smear negative & culture positive.

In 1993, WHO was confirmed that TB is a global health crisis. Statistics for TB to claim around 1.7 million lives per year. It is estimated that one-third population of world is infected with *M. tuberculosis*, with approximately 9 to 10 million new cases reported annually.[2] In 2015, RNTCP (Revised National TB Control Programme) covered a population of 1.28 billion. Total of 91,32,306 TB cases examined by sputum smear microscopy and 14,23,181 cases were registered for treatment.[3]

AIM & OBJECTIVE: In this present history detection of Mycobacterium tuberculosis complex by Line Probe Assay (PCR).

METHODS

Study design: This study was done from March 2016 to January 2017, Non- interventional approaching study of 50 suspected patients of tuberculosis has visited the TB chest Department & DOTS center in TMMC & RC.

In this study two main methods were used for the detection of tuberculosis

1. Ziehl-neelsen (Z-N) staining
2. Line probe assay

Z-N Staining method require heating of the slide for better penetration of the stain into the mycobacterial cell wall, so it is also known as Hot stain procedure. For this stain we were used 20% sulphuric acid.[4]

In Line Probe Assay, according to WHO, MTBDR assay test was performed in three separated room.

- ✓ First room - used for DNA extraction in BSL-3
- ✓ Second room – used for preparation of master mix
- ✓ Third room - used for PCR and hybridization

In the condition of sputum sample, first of all sputum sample was digested and decontaminated properly by NALC (N-acetyl L- cysteine) method. After than 500 microliters sediments was used to perform the Genotype MTBDR Plus (Hain Lifescience GmbH) assay. After DNA extraction, residual specimen were proceed for PCR and hybridization and this specimen was stored at 2-8^{0}C overnight in refrigerator.

- ❖ **STEPS: -**
  - A.) Decontamination
  - B.) DNA Extraction
  - C.) Amplification
  - D.) Reverse Hybridization

RESULTS

In this study, sputum samples which were clinically suspected with tuberculosis infection attending in TB & chest department and samples were proceed under molecular laboratory in TMMC & RC.

Table 1: Showing age range and their sex wise distribution

Age range	Male	Female	frequency	Percentage (%)
< 25	5	1	6	12
26-50	11	5	16	32
51-75	20	6	26	52
>75	0	2	2	4

On the above table, the maximum prevalence of positivity seen among the patients are males. Below the age of 25, the number of males are 5 as compare with females. Above the age of 75, the number of male is none but female were 2.

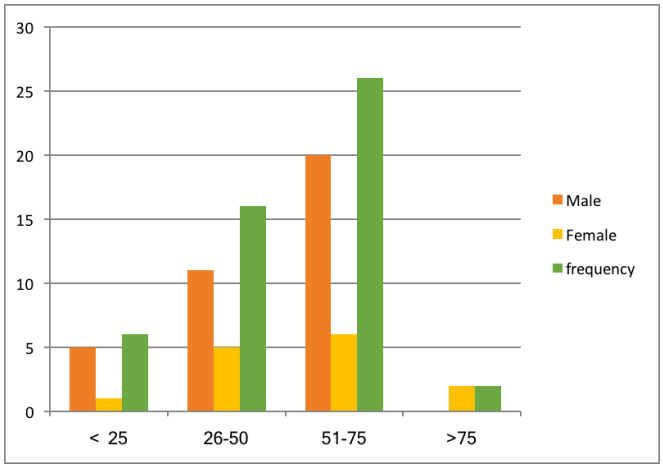

Fig 1: Graph showing the age range and the maximum information between in male and female.

Result for Z-N Staining:

50 samples were collected, out of 50 samples only 10 samples were smear positive in which 5 sample were showing (+1)) smear positive, 3 sample showing (+2) smear positive and 2 sample was (+3) smear positive based on AFB were presented at the time of microscopy. Hence 40 sputum sample were smear negative reason of the absence of Acid-Fast Bacilli.

Table 2: showing the information of the result of acid- fast staining on the basis of smear positive and negative

Total	50	100%
Positive	10	20%
Negative	40	80%

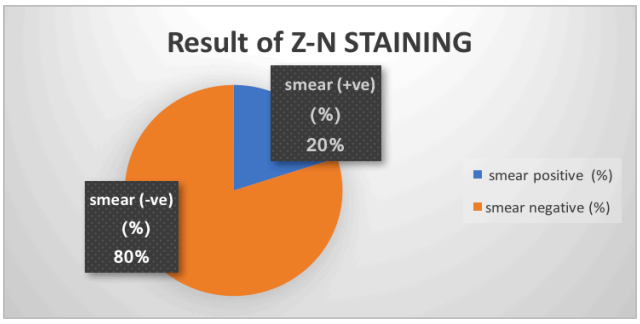

Fig 2: Pie chart showing the range of smear positive and smear negative in the presence of total number of samples.

Result of Line Probe Assay:

Result for PCR was conducted after taking the sample from DOTS centre by means of the written consent of the patients in molecular lab of T.M.M.C & R.C.

In 50 samples, out of which 10 (20%) were smear positive & 40(80%) were smear negative. Out of 10 smear positive, 9 (90%) were MTB (Mycobacterium tuberculosis) & 01 (10%) was NTM (Non-tuberculous Mycobacteria) by PCR method. Out of 40 smear negative, 30 (75%) were positive by PCR method. Out of 30, 28(93%) were MTB & 02 (7%) were NTM. Rests of the 10(25%) samples were found negative for M. tuberculosis complex.

Table 3: Result of PCR in smear negative sputum samples.

SMEAR NEGATIVE SAMPLES	40
PCR positive Samples	30
PCR Negative Samples	10

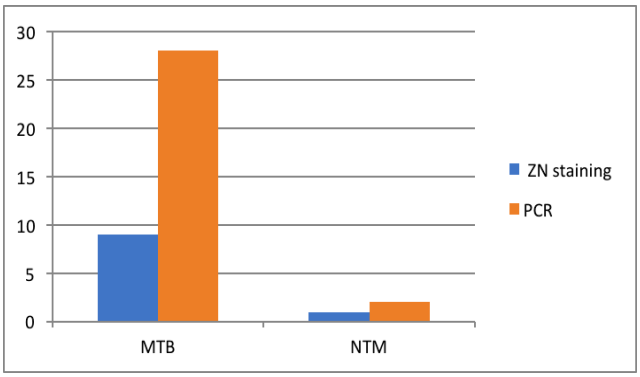

Fig 3: Bar chart showing the result of Line Probe Assay

DISCUSSION

The most conventional methods for laboratory diagnosis of M. tuberculosis including Acid- fast staining and culture method. For the improvement or rapid diagnosis including new strategies for the detection of M. tuberculosis complex are needed to help combat this fatal disease. Nucleic acid amplification used for this type of diagnostic method and they improve the detection of Mycobacterium tuberculosis complex**.** In the present year, major focus on the rapid detection of Mycobacterium tuberculosis disease which is caused by MDR or XDR resistant strains. For the accurate and early diagnostic purpose, we are used Nucleic acid amplification test like PCR. This method is more sensitive and more specific.[5-7] Pulmonary tuberculosis was found more in patients of age group 51-75 years. In the susceptible individuals, the results of cumulative effects of smoking is the respiratory problems in the association of ageing in the environmental exposure. Our study also found that patients were chronic smokers; this might be the reason behind the higher number of patients of pulmonary T.B. Moradabad area was found to be the thrust area for tuberculosis, because the population which lived in the villages had to come to the cities to earn their livelihood.[5] In this study, we were discuss that in total 50 sputum samples, only 10 (20%) samples gives smear positive and 40 samples gives smear negative by using of Z-N Staining. It is similar with the sudy of S Sankaret al.[6] in their study out of 84 patients smples, 17 (20.4%) were smear positive. And it is also similar with the study of Oyebode A. T. Alli et alin which 72 (36%) out of 200 samples were smear positive.[8]

In our study, total 50 samples were proceed. The maximum numbers of males were in the age group 51-75 years while the maximum numbers of females were in the age group 26-50 years. It is similar with the study of S Sankar et al. The average ages of males were 48 and females were 40 years.[6] Out of 10 smear positive samples, 9(90%) were Mycobacterium tuberculosis (MTB) positive and 1(10%) was Non –tuberculous Mycobacteria (NTM) positive by PCR method. This result similar with the study of Oyebode A.T. Alli et al., they found that out of 200 samples 84(42%) were positive for M. *tuberculosis* complexes.[8] On other hand, out of 40 smear neagtive samples, 30 (75%) samples were positive by PCR method and remains samples were negative. Out of 30positive samples, 28(93%) were MTB and 2 (7%) samples were NTM. This result similar to the study of Aroma Oberoi, Aruna Aggarwal that revealed by the use of PCR method they were able to detect M. tuberculosis from smear negative, out of 150 smear negative, 90(60%) were positive and 60(40%) were negative.[10] At the end discussed that Line probe assay methodology is more prone to contamination. The way around the PCR contamination in our study which neglect by used of Bio-safety cabinate level 3. Samples were proceed under the BSL-3 and its strict adherence to workflow in the laboratory helped to combat the problem of PCR contamination. [11]

CONCLUSION

We have discussed the use of molecular detection of Mycobacterium tuberculosis complex in this study. On the basis of our study, we suggested for the addition of molecular diagnosis of M. tuberculosis complex used for the diagnosis of tuberculosis especially in first visit of patients to TB and chest clinics because timely intervention is useful for the treatment of tuberculosis and reduce the transmission of this fatal disease. In our study, we concluded that molecular detection of Mycobacterium tuberculosis complex by use of Line Probe Assay methodology from clinical sputum samples has high specificity and sensitivity. The maximum prevalence of

positivity was seen among the male patients. Below the age of 25, the number of male were 5 as compared to the females. Above the 75 age, the number of male was none but females were 2. We used 50 clinical sputum samples for the detection of M. tuberculosis, out of them only 10 samples were smear positive while remaining 40 samples in which 30 smear negative samples were show positive result through PCR method. This study proved that PCR is a specific and sensitive method in comparison of sputum microscopy after staining with Z-N technique and it helpful the clinicians ability to diagnose and treat the patients on time. This will ensure early treat to patients and prevent further transmission of disease.

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