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# MOLECULAR DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX FROM SPUTUM SAMPLE OF CLINICALLY SUSPECTED PULMONARY TUBERCULOSIS PATIENTS.

Microbiology

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## INTRODUCTION:

Tuberculosis (TB) is a infectious disease caused by M. Tuberculosis. The disease primarily effects lungs and respiratory system. (1)

In the world, tuberculosis is ranked second after all infectious agent due to co-infection with HIV, leading causes of mortality and morbidity due to bacterial infection (2). The causative agent of tuberculosis the group of mycobacteria is known as the mycobacterium tuberculosis complex, mycobacterium complex consists of M. tuberculosis, M. bovis, M. microti, M. caneti and M. africanum.

In 1993, TB was declared a global health emergency by the world health organization (WHO). Statistic has put TB to claim approximately 1.7 million lives per annum (3). It is estimated that one third of the world's population is infected with M. Tuberculosis complex with around 9 to 10 million new cases reported annully (4,5). Diagnostic method are commonly based on the finding of AFB on microscopic examination of a sputum specimens, L-J culture methods and chest X-ray. All methods have some helpful for the diagnosis of tuberculosis. Z-N (Ziehl-Neelsen) staining and culture are main backbone for diagnosis of mycobacterium tuberculosis, now a methods PCR (Polymerase Chain Reaction) is a rapid diagnostic test which is used for tuberculosis that has been evaluated in a large number of studies (6,7).

The problem of tuberculosis has been exaggerated by the appearance of multi-drug and extremely drug resistance tuberculosis (8).

In India, out of a total population of more than 1 billion, approximately 2 million develop active tuberculosis and out of them a million die by the reason of tuberculosis infection (9).

## Material and Methods:

Study design: the study was conducted in molecular laboratory from January 2017 to December 2017, total 100 sputum samples were collected from suspected patients of tuberculosis attending TB & Chest department of Teerthanker Mahaveer hospital & research centre, Moradabad.

In this study mainly two methods were used for the Detection & Confirm of tuberculosis from sputum samples.

A. Ziehl-Neelsen (Z-N) staining.
B. Line probe assay (LPA).

In 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.

## In Line Probe Assay:

Firstly, 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.

**v STEPS:-**

A.) Decontamination
B.) DNA Extraction
C.) Amplification
D.) Reverse Hybridization

## RESULT AND DISCCUSION:

In this study, 100 sputum samples were collected from patients attending TB & Chest department and sample were procced in molecular laboratory of TMMC & RC.

Total sample according to Age range and sex wise distribution:

| Age range | Male | Female | Frequency | Percentage ( % ) |
|---|---|---|---|---|
| < 25 | 8 | 3 | 11 | 11% |
| 26- 50 | 21 | 14 | 35 | 35% |
| 51-75 | 34 | 15 | 49 | 49% |
| >75 | 3 | 2 | 5 | 5% |

**Table 1: showing age range and sex wise distribution**.

In the table, the maximum prevalence of positivity seen among the males patients. Below the age of 25, the number of males are 8. Above the age of 75. The number of males is 1 but female were 4.

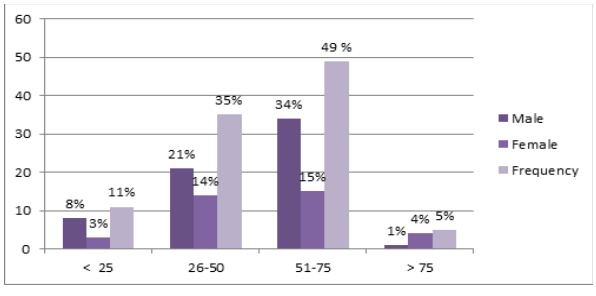



**Figure 1: Graph showing the age and sex wise distribution.**

## RESULT FOR Z-N STANING:

100 samples were collected, out of 100 samples only 20 samples were smear positive in which 9 samples are showing (1+), 7 samples are showing (2+) and 4 samples was (3+) smear positive on the basis of AFB presented

in the time of microscopy. Hence 80 samples were smear negative due to the absence of Acid-Fast-Bacilli in sputum samples.

| | | |
|---|---|---|
| Total sample | 100 | 100 % |
| Smear positive | 20 | 20 % |
| Smear negative | 80 | 80% |

**Table 2 :- Showing the result of Z-N staining on the basis of smear positive and negative.**

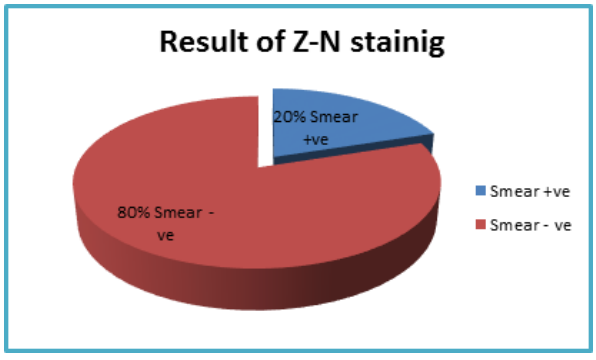



**Fig 2: pie chart Showing the smear positive and smear negative in the presence of total number of samples**

**RESULT OF LINE PROBE ASSAY:**

During the study period,100 sputum samples were collected and procced in molecular laboratory of TMMC & RC.

In 100 samples, out of which 20 (20%) were smear positive and 80 (80%) were smear negative. Only smear negative samples were further studies by Line Probe Assay. Out of 80 smear negative, in which 12 (15%) were MTB (Mycobacterium Tuberculosis) positive and 68 (85%) samples was MTB negative by PCR methods.

| | | |
|---|---|---|
| Total Smear Negative Samples | 80 | 100% |
| PCR Positive Samples | 12 | 15% |
| PCR Negative Samples | 68 | 85% |

**Table 3 : Result of Line Probe Assay in Smear negative samples.**

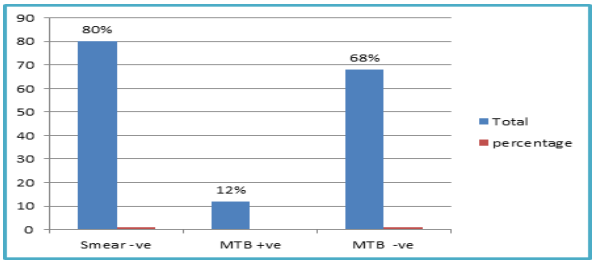



**Fig 3: Bar Chart Showing the result of Line Probe Assay**

**Comparision Between Z-N staining and Line Probe Assay:**

In this study, total 100 sputum samples were proceed, out of 100 samples 20 (20%) samples were smear positive based on Z-N staining. On the basis of Line Probe Assay out of 80 smear negative samples in which 12 (15%) samples were MTB positive and 68 (85%) samples was MTB negative. LPA show highly sensitivity & confirmatory method.

| Result | Z-N staining | Line Probe Assay |
|---|---|---|
| Positive | 20 | 12 |
| Negative | 80 | 68 |

**Table 4 : showing comparative data between Z-N staining & LPA**

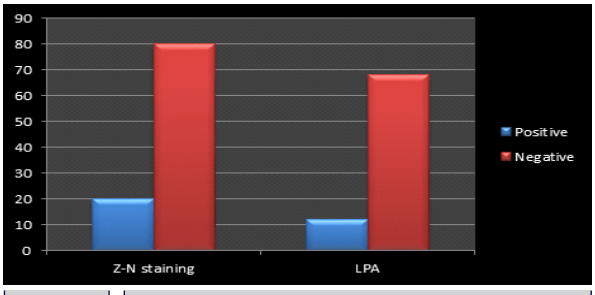



**Fig 4 : chart show result for the comparison of Z-N staining and Line Probe Assay.**

Strip showing Tuberculosis (TUB) band which the presence of M. Tuberculosis complex (MTB) and other band described sensitivity and resistance for rpoB, KatG and inhA gene mutation

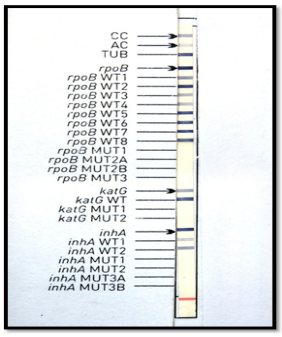
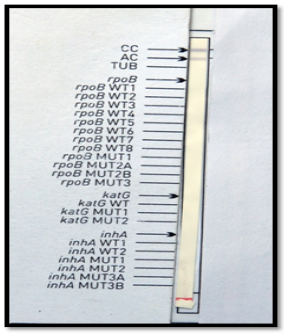



**Figure 5 : strips Tuberculosis (TUB) band showing presence of (MTB) M. tuberculosis complex**




- **Fig6 : Strips Tuberculosis (TUB) band showing absence of M.tuberculosis complex (MTB).**
- **Conjugate Control (CC):** A line develop in this zone, documenting the Efficiency of conjugate binding and substrate reaction.
- **Amplification Control (AC)** : Development of this band excludes mistakes during set up and performance of amplification reaction.
- **Tuberculosis (TUB) band**: positive band means member of MTB complex present, whereas in case of negative TUB band and positive resistant pattern then test should be repeated.
- **Mutation Probe (MUT)**: these Probes detect a number of the mainly universal resistance mediating mutations.
- **Locus Control (rpoB, KatG, inhA)**: for respective locus these zone detect a gene region specific.

**DISCUSSION**:

Infection of mycobacterium tuberculosis complex is one of the most important global public health problem. In 2016, there were an estimated 10.8 million new TB cases worldwide and 1.4 million TB death in 2015. TB Remained one of the top 10 causes of death worldwide in2015 (10).

The improvement or rapid diagnosis of M. tuberculosis complex including new strategies, nucleic acid amplification and CBNAT. This method is more sensitive and more specific (11).

In our study, total 100 sputum samples were processed. The maximum number of males (49%) were in the age group 51 to 75 year. While the maximum number of females (35%) were in the age group 26 to 50 . it is similar with the study done by S. Sankar et al, the average ages of males were 48 and females were 40 years (12,).

In this study, we were observed that in total 100 sputum samples, in which 20 (20%) samples smear positive and 80 (80%) samples smear negative by using Acid-Fast staining. It is similar with the study of S. Sanker et al , in their study out of 84 samples, 17 (20.4%) were smear positive (12,13).

Out of 80 smear negative samples, 12 (15%) samples were MTB positive and 68 (85%)samples were MTB negative by Line Probe Assay methods. This results similar to the study of Kumar R.et al, in their study out of 904 smear negative specimens 80 (9%) MTB positive and 707 (44%) were MTB negative on the basis on use of Line Probe Assay (LPA) (14).

The present study we had made an attempt to know the prevalence of pulmonary tuberculosis in smear negative suspected cases in western U.P. by using Line Probe Assay. LPA is a faster and more sensitive and more specific method for diagnosis of Mycobacterium tuberculosis when compared with Acid-Fast staining. And it further detects and early diagnosis of Drug- resistance tuberculosis.

## CONCLUSION:

This study Proved Line Probe Assay (LPA) that is Rapid and Highly specific and sensitive method for Detection Mycobacterium Tuberculosis complex and Drug- Resistance TB from sputum samples. In our study 100 sputum samples we used for the detection of mycobacterium tuberculosis, in 100 samples, out of which 20 samples smear positive and 80 samples were negative. Only smear negative samples were further studied by Line Probe Assay (LPA). Out of 80 smear negative, in which 12(15%) were MTB positive and 68 (85%) samples was MTB negative by PCR methods.

On the basis of this study we suggest that molecular detection of M. tuberculosis complex should be used for diagnosis of tuberculosis on the first visit of patient to DOTS and TB & Chest department because, timely treatment of TB can reduced spread of this organisms.

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