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Analysis of Different Tests for Diagnosis of Pulmonary Tuberculosis

Anita Kulkarni[1], Dr. Kirti Dubey[2], Dr. Pallavi Wanjari[3], Dr. Yagnesh Thakar[4]

[1, 2]Sevada lMahila Mahavidyalaya, Nagpur, Maharashtra, India

[3, 4]Vishakha Clinical Microbiology Laboratory, Nagpur, Maharashtra, India

Abstract: ***For the diagnosis of pulmonary tuberculosis phenotypic and genotypic tests were analysed. On 269 pulmonary specimens comprising of Sputum (139), Bronchoalveolar lavage (B. A. L.) (90), Pleural fluid (21) and Endotracheal secretions (E. T.) (19) ZeihlNeelson (ZN) stain, Automated TB culture, Line Probe Assay (LPA), Nucleic Acid Amplification Test (NAAT) (TrueNat) and Real Time PCR (RTPCR) (Hi media) were performed. A total of 123 (45.72%) specimens were positive either by single or multiple tests together. Of these highest positivity was seen in 72 (58.54%) sputum specimens followed by 35 (28.46%) B. A. L., 9 (7.32%) pleural fluids and 7 (5.69%) E. T. secretions. Highest positivity amongst the tests was seen with 121 (98.37%) NAAT test, 119 (96.74%) Culture and 116 (94.31%) RTPCR while it was low with 83 (67.48%) ZN stain and 81 (65.85%) LPA test. All five tests were positive in 77 (62.60%) and Culture, NAAT and RTPCR were simultaneously positive 36 (29.27%). Molecular tests of NAAT and RTPCR are most favored tests for diagnosis of TB which can be substantiated by Culture if required.***

Keywords: Mycobacterium tuberculosis, NAAT, RTPCR, Line probe Assay, Sputum

1. Introduction

A total of 1.5 million people died from TB in 2020 (including 214 000 people with HIV). Worldwide, TB is the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS). In 2020, an estimated 10 million people fell ill with tuberculosis (TB) worldwide.5.6 million Men, 3.3 million women and 1.1 million children. TB is present in all countries and age groups. But TB is curable and preventable (1). At the turn of the century, it was widely recognized that an accurate point-of care test for TB was required to make significant reductions in the pandemic (2).

TB mainly affects the lungs (pulmonary TB), however, it can affect other parts of the body also (extra pulmonary TB). Although TB is a preventable and curable disease, failure to detect the disease early is one of the major bottlenecks to TB control (3). Fast and effective microbiological diagnosis is essential to control the spread of TB. The most common diagnostic method is sputum smear microscopy with culture methods or rapid molecular testing used in countries with advanced laboratory facilities. Although a large number of tests are available each test has its merits and demerits. Recently developed molecular tests have definite advantage of speed, sensitivity and specificity (4).

In the present study performance of different tests including phenotypic tests like Ziehl Neelson (ZN) stain, Culture, and genotypic tests of Line probe assay (LPA), Cartridge based Nucleic Acid Amplification Test (NAAT) (Trunat, Molbio) and Real time PCR (RTPCR) test MBPCR017 (Hi-media) tests were evaluated for diagnosis of tuberculosis.

2. Material and Methods

The study comprises of different pulmonary samples obtained serially from patients suspected for Tuberculosis during the period 2017-2021.

A total of 269 pulmonary specimens were appropriately collected and received in the laboratory for the diagnosis of tuberculosis which comprised of 139 sputum specimens, 90 Broncho alveolar lavage (B. A. L.) specimens, 21 pleural fluids and 19 Endotracheal (E. T.) secretions.

The specimens were decontaminated using the standard method (5)and tested by Ziehl Neelson (ZN) staining, Culture, Line Probe Assay (LPA), N. A. A. T. test (Truenatby Mol Bio) and RT PCR (Hi Media).

Z. N. Stain: Smears were prepared from the decontaminated specimens, heat fixed and stained by the standard Z. N. Staining method (6).

Culture: The decontaminated specimens in the volume of 0.5 ml each were inoculated in the BacT Alert MP bottles and loaded in the BacT-Alert 3D automated culture system and incubated until flagged positive by the system or up to 42 days if negative.

The bottles flagged positive were further tested for confirmation of M. tuberculosis by drawing 0.5 ml of culture medium by the syringe and needle, performing Z. N. stain for Mycobacterium species and further confirmation of species by Immunochromatographic method (5).

Line Probe Assay: The LPA test comprises of PCR followed by reverse Hybridization. The test was performed using the kits manufactured by Hain Laboratories, Germany. The PCR was done using thermocycler (Eppendorf) for 30 cycles. Reverse hybridization was performed in the Twincubator (HainLifesciences). The tests were performed according to the manufacturer's instructions.

N. A. A. T. Test: The Nucleic Acid Amplification Test (MolBio) is a real time cartridge based PCR test and it was performed as per manufacturer's instruction.

R. T. PCR Test: The real time PCR test was done using the MBPCR017 kits (Hi Media) and loaded in the Real Time PCR system (Hi Media). The tests were done according the manufacturer's instructions.

3. Observation

Study comprises of total of 269 specimens obtained from patients with suspected pulmonary TB. Out of these 152 (56.51%), were from males and 117 (43.49%) from females.

Of the total respiratory specimens, tuberculosis was detected in 123 (45.72%) specimens either by any single test or more tests together. The specimen wise distribution of positive and negative test results is shown in Table no.1
Out of 123 positive samples, 74 (60.16%) were male and 49 (39.84%) were female.

Out of the five tests included in the study N. A. A. T. tests showed the highest positivity followed by culture. The positivity of different tests is shown in Table 2.

The positivity of different tests either alone or together in different combinations revealed eight different combinations. Their pattern is shown in Table 3.

Table 1: Specimen wise distribution of all positive and Negative samples

Specimens	Negative, N=146	Positive, N=123
BAL	55 (37.97%)	35 (28.46%)
ET	12 (8.22%)	7 (5.69%)
PLEURAL FLUID	12 (8.22%)	9 (7.32%)
SPUTUM	67 (45.89%)	72 (58.54%)

Table 2: Shows Positivity by different Tests

Tests	Positive	% (N=123)
ZN	83	67.48
CULTURE	119	96.74
LPA	81	65.85
NAAT	121	98.37
RTPCR	116	94.31

Table 3: Test wise Positivity, either individually or combined.

Tests	Total	%N=123
ZN	1	0.81
CULTURE	1	0.81
ZN+NAAT+RTPCR	1	0.81
ZN+CUL+LPA+NAAT	2	1.63
ZN+LPA+NAAT+RTPCR	2	1.63
ZN+CUL+LPA+NAAT+RTPCR	77	62.60
CUL+NAAT	3	2.44
CUL+NAAT+RTPCR	36	29.27
TOTAL	123	100

4. Discussion

The correct and early diagnosis of tuberculosis definitely helps in proper treatment and elimination of TB. In the present study as many as 45.72% specimens were positive for TBeither by single test or more tests together from different pulmonary specimens(7).

The positivity was more in males as compared to females. Similar results are reported in other studies also. (8), (9).

Of the 123 positive specimens. highest positivity was seen byNAAT (98.37%) followed by Culture (96.74%) and RTPCR (Himedia) (94.31%) while the positivity of ZN stain (67.48%) and LPA was low (65.53%) Many other studies have reported molecular diagnostic tests to be most useful for diagnosis of tuberculosis. (10)(7)(11)It is well known that sensitivity of ZN stain is quite low. Likewise LPA is generally used in smear positive cases. Its positivity in smear negative cases is very low.

The data about either individual or combined positivity of test results revealed eight different combinations with highest positivity seen in 62.60% cases in which all the test are positive. Hence it may be considered that in 62.60% cases any of the tests can be used for diagnosis. While in about 29.27% cases Culture, N. A. A. T. and RTPCR were simultaneously positive. Hence in about 91.87% cases diagnosis can be made by any one of the test of culture or N. A. A. T. or RTPCR (Himedia).

However culture is time consuming. But it can give the status of live organism. While the molecular tests of N. A. A. T. and RTPCR are fairly rapid. Amongst the two N. A. A. T (Truenat) is less laborious than RTPCR.

In view of this it can be recommended thatN. A. A. T. is a preferred single test for diagnosis of Tuberculosis due to its speed, simplicity and very high sensitivity.

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