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Original Research Article

To Evaluate the Sensitivity, Specificity of Real Time PCR (TrueNAT) Assay in Case Detection of Mycobacterium Tuberculosis in Presumptive Pulmonary Tuberculosis Cases

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Abstract

Aim of Study: To evaluate the Sensitivity, Specificity of Real-time PCR (TrueNAT) assay in case detection of Mycobacterium Tuberculosis in Presumptive Pulmonary Tuberculosis Cases(Culture Positive).

Material And Methods: This was a prospective, hospital based cross sectional study carried out in SCB Medical College and Hospital, Cuttack, Odisha for a period of one year, i.e from Sept 2019 –August 2020, on 50 Presumptive Pulmonary tuberculosis patients visiting inpatient and out-patient department of Pulmonary Medicine. Statical analysis of the data was conducted with Statistical Package for the Social Sciences (SPSS) VERSION 20.0.

Results: Considering culture as the gold standard test the results of TrueNAT (RT PCR) was compared for Pulmonary tuberculosis specimens in present study. Performance of Real-time PCR (TrueNAT) assessed against Culture in this study, Sensitivity was 92.11%, Specificity was 91.67%.

Conclusion: Our study concludes that Real Time PCR (TrueNAT) has shown good results and has high sensitivity and specificity for detection of Mycobacterium tuberculosis in Presumptive Pulmonary tuberculosis patients.

Keywords: TrueNAT, Pulmonary Tuberculosis, Real Time PCR.

Introduction

Tuberculosis (TB) causes the highest number of deaths globally, attributable to a curable infectious agent [1], despite the availability of potent anti-TB medication. Reduction in TB-related morbidity and mortality is impeded by the lack of rapid and cost-effective diagnostic tests that are implementable in resource-limited settings.[2]

Tuberculosis is known since ages. It is presumed that the given Mycobacterium

originated more than 150 million years ago. Tuberculosis was recorded in Egyptian Mummies way back in 5000 BC. The first written account of tuberculosis is found in the Vedas. The most ancient of them all, Rig Veda. In 1500 BC Rig-Veda Tuberculosis was described as Yakshama. (Agarwal Y *et al*) [3] In 1300 BC it was recorded in the laws of manu in India. Mycobacterium tuberculosis is one of the world most important public health challenges. It is a global epidemic with cases from almost every country, which has affected the health and welfare of the nations and increased mortality and morbidity.

Demonstration of acid-fast bacilli (AFB) in a smear made from a clinical specimen provides a preliminary diagnosis of mycobacterial disease, while the isolation of mycobacteria on culture provides a definite diagnosis of tuberculosis or disease due to mycobacteria other than M. tuberculosis (MOTT bacilli) or non-tuberculous mycobacteria (NTM). As much as 50-60% of AFB culture-positive clinical specimens may fail to reveal AFB on smear made from the specimen. As a consequence, culture techniques play a key role in the diagnosis of mycobacterial disease.[4]

TrueNAT is a MEMS based (Micro Electro Mechanical system based) Real time micro Polymerase chain reaction (PCR) device. PCR is the gold standard in infectious disease diagnosis because of its high level of sensitivity and specificity to the disease case. True NAT is a portable and battery operated cost-effective device designed to be used by minimally skilled technicians. It also offers rapid detection time of 45-60 minutes.[5]

Material and Methods

Duration of Study: Study was carried out for a period of one year, i.e from Sept 2019 – August 2020.

Type of Study: This was a prospective, hospital based cross sectional on 50 Presumptive Pulmonary tuberculosis patients visiting inpatient and out-patient department of Pulmonary Medicine.

Inclusion Criteria:

- Patient's attending OPD and IPD of age of > 15 years in the Dept. of Pulmonary Medicine.
- Patient's with clinical suspicion of pulmonary tuberculosis including symptoms of cough with expectoration for >2 weeks, fever for >2 weeks, weight loss, loss of appetite and hemoptysis, and any abnormality in chest radiograph.
- Immunocompromised patients screened for Pulmonary Tuberculosis.

Exclusion Criteria:

- Patients already diagnosed as microbiologically confirmed Pulmonary TB or Clinically diagnosed Pulmonary TB on Anti tubercular Treatment.
- Pediatric patients and critically ill patients, those are not able to expectorate the sputum.
- Patients having Extra Pulmonary Tuberculosis.
- Patients not giving consent.

Methodology

Sputum specimen collected in a 50 ml centrifuge tube, with a screw cap. NaOH-NALC-sodium citrate solution added in a volume equal to the quantity of specimen. Then inoculation of MGIT medium is done. Inoculated MGIT (7mL) tube is entered in the BACTEC MGIT 960 instrument after scanning each tube. Mycobacterial growth appears granular and not very turbid while contaminating bacterial growth appears very turbid. Growth, especially of the *M. tuberculosis* complex, settles at the bottom of the tube.

Real-time PCR assay done by battery-operated, portable micro-RT- PCR device, the TrueNAT. MTB,RT-PCR micro device, as well as Trueprep MAG for extraction of DNA directly from samples for early detection of Mycobacterium tuberculosis performed in the Molecular laboratory of the Department of Biochemistry.

Statistical Analysis

Statistical analysis was performed using (Statistical package for social science) SPSS 20 software and the analyzed data was expressed in percentages. P-value equal to or less than 0.05 was considered to be significant.

Table 1: Comparison of TrueNAT against Culture

Test	Culture Positive (n=38)	Culture Negative (n=12)	χ^2	p-value
TrueNAT Postive (n=36)	35	1	31.74	0.0001
TrueNAT Negative (n=14)	3	11		

On comparing TrueNAT for M,Tb with Culture, 36 patients were positive for TrueNAT for M.Tb out of which 35 positive for Culture, 1 patient was Culture negative.

14 patients were Negative for TrueNAT for M.Tb, 3 patients were Culture positive and 11 patients were Culture negative among them.

The Result is showing statically significant association at $p < 0.05$

Table 2: Performance of TrueNAT for detection of PTB with respect to Culture

Characteristics	Value	95% CI
Sensitivity	92.11%	78.62% - 98.34%
Specificity	91.67%	61.52% - 99.79%
PPV	97.22%	84.24% - 99.57%
NPV	78.57%	54.98% - 91.67%

Performance of TrueNAT assessed against Culture Sensitivity was 92.11%,Specificity was 91.67%,Positive Predictive value was 97.22% and Negative Predicitive value was 78.57%.

Discussion

In present study among comparing diagnostic modalities for detection of Mycobacterium Tuberculosis TrueNAT for M.Tb, and Culture tested positive for 36(72%) and 38(76%) patients respectively among 50 patients and TrueNAT for M.Tb and Culture tested negative for 14(28%) and 12(24%) patients respectively.

Performance of Real time PCR (TrueNAT) assessed against Culture in this study, Sensitivity was 92.11%, Specificity was 91.67%. The sensitivity and Specificity of RT-PCR(TrueNAT) for diagnosis of Pulmonary tuberculosis is 93.1% and 72.5% as reported in Mangayarkarasi *et al*.[6]

In a study Kim MJ *et al*.[7] Sensitivity and specificity of Real time PCR was 80.0% and 85.7% respectively. According to Nikam *et al*.[8] Sensitivity and specificity of Real time PCR(TrueNAT) was 94.70% and 52.85% respectively. The diagnostic sensitivity of Real time PCR (TrueNAT) showed 92.11% which is quite comparable to Mangayarkarasi *et al*.[6] and Nikam *et al*[8] results.

Conclusion

Our study concludes that Real Time PCR (TrueNAT) has shown good results and has high detection rate of Mycobacterium tuberculosis in Presumptive Pulmonary tuberculosis patients and microscopy can be

replaced by TrueNAT as POC (Point of care) TB diagnostic tests.

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