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Diagnosis of Extra pulmonary Tuberculosis and detection of Rifampicin Resistance in extra pulmonary tuberculosis suspects by Gene Xpert MTB/RIF in South Gujarat.

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Abstract

Background: Tuberculosis (TB) is the world's leading infectious cause of death. Extra pulmonary Tuberculosis accounts for 15% of TB cases, but the proportion is increasing.[1]WHO estimates a Rifampicin resistance rate of 3.5% in new cases while 18% in treated cases.[2]The paucibacillary nature of disease and non-uniform distribution of microorganisms may lead to false negative results.[4]That's why rapid and effective diagnosis of EPTB is required.

Methods: It is retrospective study analyzing the data of suspected Extra pulmonary Tuberculosis sample received at T.B.I.R Laboratory, Department of Microbiology, GMC, Surat from various areas of South Gujarat region during 1st February 2022 to 31st May 2022 (4 Months).A total of 160 suspected EPTB sample were received and tested by Gene Xpert MTB/RIF.

Results: Out of the 160 samples tested by Gene Xpert MTB/RIF, 43 samples (27%) were detected to be positive for EPTB, and 117 samples (73%) were found to be negative for EPTB. Diagnostic results for Rifampicin resistance by Gene Xpert MTB/RIF on the 43 Positive EPTB samples analyzed showed a Rifampicin resistance rate of 9.3% (n = 4). Two samples were rifampicin indeterminate which required repeat testing on 2nd sample. Out of 4

rifampicin resistance detected samples 3 were pus samples and 1 was BAL (Bronchoalveolar lavage).

Conclusion: Diagnosis of EPTB poses a major challenge due to difficulty of access to specific sampling sites results in paucibacillary samples, which reduces the sensitivity of conventional diagnostic tests.GeneXpert MTB/RIF detect M. tb and rifampicin resistance directly from clinical specimens, therefore shortens the turnaround time, which significantly improve morbidity and mortality due to EPTB.

Keywords: Extra pulmonary TB, Rifampicin resistance, Rapid diagnosis of EPTB, Gene Xpert MTB/RIF

Introduction

Tuberculosis (TB) is an ancient disease known to mankind which found in humans dating from at least 5000 BCE and still tuberculosis remains one of the leading causes for morbidity and mortality in the world [11]. Globally, according to 2017 World Health Organization (WHO) report that there is an estimated 10.4 million TB cases and 1.6 million TB deaths in 2016, which is much higher than 2013 estimate (9.0 million TB cases and 1.5 million TB deaths) [12].WHO estimates a Rifampicin resistance rate of 3.5% in new cases while 18% in treated cases. Moreover, the rates vary according to the region and tubercular endemicity as India has 23% of global annual rate [2,11].

TB is a contagious disease, caused by the organism Mycobacterium tuberculosis (MTB), predominantly involves the lungs [10]. Extra pulmonary tuberculosis (EPTB) refers to TB occurring within a location in the body other than the lungs (e.g., meninges, lymph nodes, pleura, genitourinary tract, joints, and bones) [13]. The annual global incidence of EPTB has been increasing in the last decade due to the changing TB control practices, spread of HIV (human immunodeficiency virus)and population growth [4].It was believed that pulmonary TB constitutes around 85% of total TB cases, whereas the remaining 15% are EPTB cases. But current data from around the world show a huge variation in the proportion ranging from 15% to 53% [10]. The proportion of EPTB is 14% reported by WHO in 2017 worldwide [2].

Mycobacterium Tuberculosis Bacilli (MTB) load is generally very low in non-respiratory sample; because of paucibacillary sample, reduces the sensitivity of conventional diagnostic test with strongly affecting the sensitivity of acid-fast microscopy [2,6,15].Diagnosis of EPTB is difficult and non-specific as various clinical presentations and varied and non-specific symptoms except some time nodal enlargement [10,11].TB control programs had accorded lesser importance to EPTB as it was believed to be non-contagious, but there are studies which show the communicable nature of EPTB. So, early diagnosis of EPTB is very important, as late diagnosis results in morbidity, increased mortality and disease sequelae [10].

EPTB patient are more likely to have negative sputum smear result because many EPTB cases do not have direct lung involvement [4].Phenotypic method have disadvantage such as operator dependency, the need for specialized laboratories and the long delay in reporting results (up to 10 days for solid medium techniques) result in high cost of realization [2].The molecular test Gene Xpert MTB/RIF has dramatically improved the diagnosis of TB by reducing the time to results which

directly impacted the patient's isolation period and decreasing the time to initiate anti-TB drugs [3].

Foundation for Innovative New Diagnostics (FIND) introduced cartridge-based nucleic acid amplification assay (Gene Xpert MTB/rifampicin [RIF]) [15]. The test runs on the GeneXpert platform (Cepheid, Sunnyvale, CA) which use a disposable plastic cartridge with all required reagents [7].The cartridge-based nucleic acid amplification test (CBNAAT) assay is a real-time polymerase chain reaction (PCR) cartridge-based assay carried out in a fully automated manner, including bacterial lysis, nucleic acid extraction and amplification, and amplicon detection to detects MTB directly from clinical samples as well as RIF resistance which is the surrogate marker of MDR-TB conferring mutations in 81 bp RIF resistance determining region (RRDR) of the rpoB gene, which codes for a beta subunit of RNA polymerase of MTB, is the genetic basis of rifampicin (RIF) resistance; which simultaneous detect Mycobacterium tuberculosis complex and rifampicin resistance from biological specimen samples within 2 hours [10,15].

The Gene Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) has been endorsed by the World Health Organisation (WHO) in December 2010. The WHO had issued policy recommendations to perform the CBNAAT assay on respiratory samples in 2011 [8,10]. In 2014, The Gene Xpert MTB/RIF assay had been strongly recommended by WHO for testing of non-respiratory specimens for EPTB diagnosis from suspected patients and detect multidrug-resistant TB over the conventional tests [15].Meta-analysis published by WHO showed the sensitivity of CBNAAT in EPTB like lymph node TB, CNS TB, pleural TB are 84.9%, 79.5%, and 43.7%, respectively compared to culture [8].

Burden of EPTB and drug resistance vary from place to place, therefore the aim of our study is toDiagnose of EPTB based on site of disease in people suspected to have EPTB and detect Rifampicin resistance in sample that are positive for EPTB.

Materials & Methods

Type and place of study: Study was a retrospective observational record-based analysing the data of suspected extra pulmonary TB sample received in the T.B.I.R Laboratory attached to, Department of Microbiology, GMC, Surat.

Study period: From 1st February 2022 to 31st May 2022 (4 Month).

Sample collection: A total of 145 clinically suspected EPTB samples like Lymph-node, cerebrospinal fluid, tissue biopsies, pus, aspirates, pleural punctures or biopsies, BAL and other localizations were received in T.B.I.R lab from various department of our hospital.

Inclusion criteria: Extra pulmonary samples (Lymph-node, cerebrospinal fluid, tissue biopsies, pus, aspirates, pleural punctures or biopsies, BAL) from all the age group irrespective of gender.

Exclusion criteria: Patients who already have pulmonary TB is not included in our study.

Sampling method: Extra pulmonary samples (Lymph-node, cerebrospinal fluid, tissue biopsies, pus, aspirates, pleural punctures or biopsies, BAL) were collected in special, plain, universal 50 ml clear plastic container with cap (falcon tubes) under aseptic conditions.

Sample from non-sterile sites needs prior treatment like digestion, decontamination and concentration. A different pre-treatment was adopted according to the sample type; non-sterile samples were decontaminated with standard NALC NaOH (1% final concentration) procedure and concentrated by centrifugation [9].All

samples underwent fluorescence microscopy for acid-fast bacilli and culture on solid (Lowenstein–Jensen) and liquid (MGIT, Becton Dickinson Biosciences, MD, USA) media. Samples were also tested with the GeneXpert MTB/RIF assay with adherence to the manufacturer's protocol. GeneXpert MTB/RIF automated molecular assay for rapid diagnosis of TB and detection of Rifampicin resistance.

In 5ml of Pre-treated sample buffer solution was added, then the mixture is loaded to cartridge which were processed by GeneXpert MTB/RIF assay (Cepheid-Sunnyvale-USA), as per the guidance document given by Central TB division, Government of India. The results can be read as MTB detected, MTB not detected, RIF resistance detected; RIF resistance not detected; RIF resistance indeterminate; or invalid/error [15].

Results & Discussion

GeneXpert MTB/RIF is a WHO recommended rapid, automated, nucleic acid amplification assay [1].Phenotypic methods have disadvantages such as operator dependency, need skilled laboratory technicians, appropriate bio-safety conditions (BSL-3) and the long delay in reporting results, but GeneXpert MTB/RIF simultaneous detection of Mycobacterium tuberculosis complex and rifampicin resistance from specimens within 2 hours [2,4].

EPTB detection by GeneXpert MTB/RIF (CBNAAT)

In this study, a total of 160 patient samples were analyzed. Out of the 160 samples tested by GeneXpert MTB/RIF, 43 samples (27%) were detected to be positive for EPTB, and 117 samples (73%) were found to be negative for EPTB which is shown in (Figure 1).

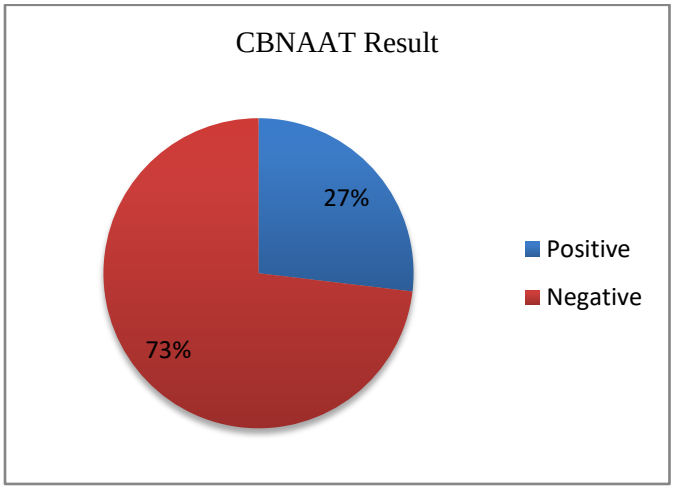

Figure 1: CBNAAT Results

Compare to our study, A study conducted by Yadhav M L K et al. "showed among 281 samples, 67(23.8%) were positive and 214(76.1%) were negative for MTB" [15], which is almost similar to our result. A study conducted by Aainouss A et al. "showed out of 301 sample, 35(11.6%) came positive for EPTB by GeneXpert and 266(88.4%) were negative for EPTB" [14].

Rifampicin resistance rate

Diagnostic results for Rifampicin resistance by GeneXpert MTB/RIF on the 43 EPTB Positive samples analyzed showed a Rifampicin resistance rate of 9.3% (n = 4) shown in (Figure 2). Two samples were rifampicin indeterminate which required repeat testing on 2nd sample. Out of 4 rifampicin resistance detected samples 3 were pus samples and 1 was BAL.

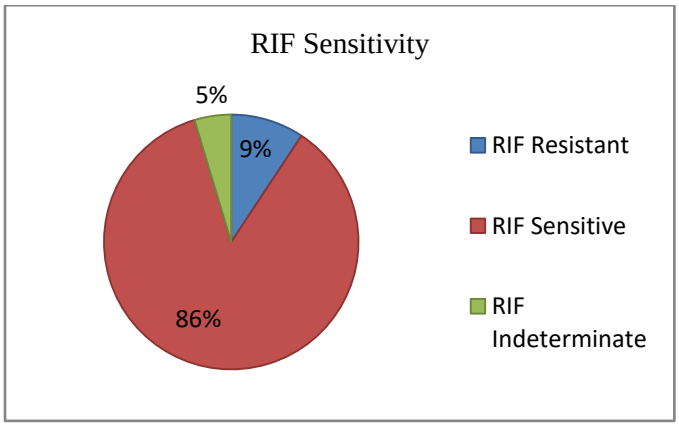

Figure 2: RIF Resistance

In Compare to our study, Yadhav M L K et al. study "has shown Out of 67 positive EPTB, 66(98.5%) were MTB-positive/RIF-resistance Negative and 1(1.49%) was MTB- positive/RIF-resistance Positive"[15].

EPTB Positivity according to age group

From a total of 160 patients initially included in the study, 16% were children (0-15 Year of age), 68% (15-45 year of age), 16% (>45 year of age). Of all patients, 68% were adolescent and working age group presumed to have EPTB. The description of the EPTB occurrence according to age group has shown in (Figure 3).

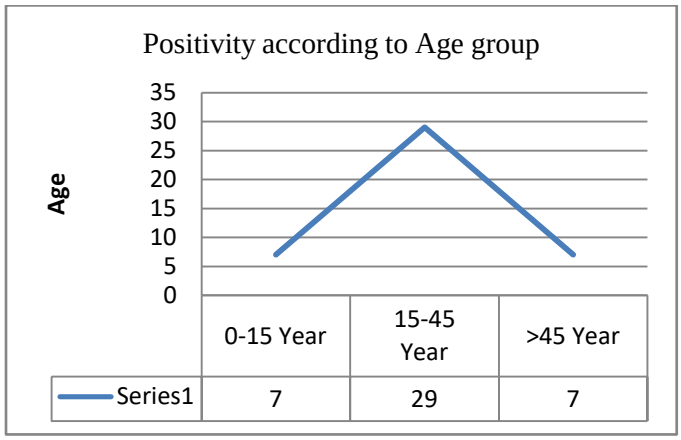

Figure 3: EPTB Positivity rate according to age group

Similar to our study, study conducted by Yadhav M L K et al. "showed Maximum number of MTB was detected in the age group of 20-30 years (n=23, 34.3%)" [15]. In Yeshi Metaferia et al. study "45.6% of study participants were in the age ranging from 31 to 50 years" [12].

Detection of EPTB in various samples:

Out of 160 Sample, 43 samples were detected MTB genen which gives positive result for EPTB by GeneXpert. Sample which came positive for EPTB by Gene Xpert are Pus, Lymph-node, BAL, Tissue & Biopsy and Fluid. Out of 67 Pus sample 27 were positive, out of 19 Lymphnode sample 7 were positive, out of 4 BAL sample 3 were positive, out of 27 tissue biopsy 4 were positive, out of 2 fluid sample 2 were positive for EPTB. It is shown in (Table 1). Out of 2 fluid sample received 2 Fluid sample were came positive for EPTB, 75% BAL sample were positive, 40% Pus sample, 37% Lymph node and 15% Tissue biopsy sample were positive compare to total received that sample.

From 27 Pus positive 3 were RIF Resistant, and out of 3 BAL positive 1 was RIF Resistant.

Sample	Total	Positive	%
Pus	67	27	40%
Lymph node	19	7	37%
BAL	4	3	75%
Tissue & Biopsy	27	4	15%
Fluid	2	2	100%

Table 1: Detection of EPTB from Sample

EPTB detection rate in sample vary from different study. A study conducted by Nishal, N et al. "showed The most common EPTB found in our study population was lymph node TB (48.1%) followed by TB pleural effusion (15.4%), abdominal TB (14.4%), bone and joint TB (13.5%) and central nervous system (CNS) TB (4.8%)" [10]. In Yeshi Metaferia et al. study, "the most common suspected Extra pulmonary sites were meninges, pleural, peritoneal, and lymph nodes in decreasing order. However, TB disease detection rate was highest among patients suspected of having TB lymphadenitis (33.3%) followed by pleural effusion (11.9%)" [12].The Mycobacterium tuberculosis complex detection rate was 33.3% for lymph node aspirates and 11.9% for pleural fluid [12]. A study conducted by Seo et al. "showed specimens (83.1% in lymph node aspirates, 71.1% in CSF, and 94.6% in bone or joint tissue)" [13]. A Yadhav M L K et al. study has shown "Out of 281 samples received, 73(25.97%) were of FNAC, 115(40.92%) Pleural fluid, 26(9.25%) Ascitic

fluid, 36(12.8%) CSF, 22(7.82%) Pus and 9(3.2%) were aspirates (other body fluids like synovial fluid, vitreous humour etc.)" [15].

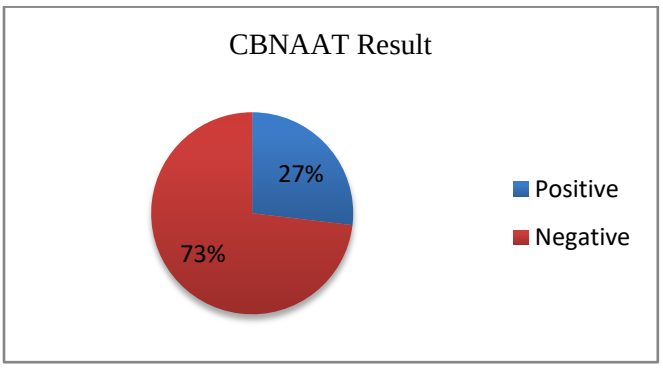

Figure 4: CBNAAT Results

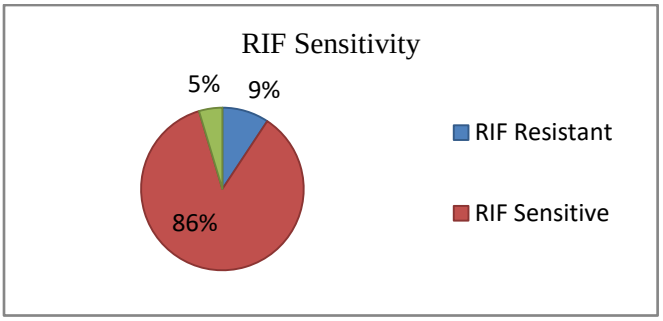

Figure 5: RIF Resistance

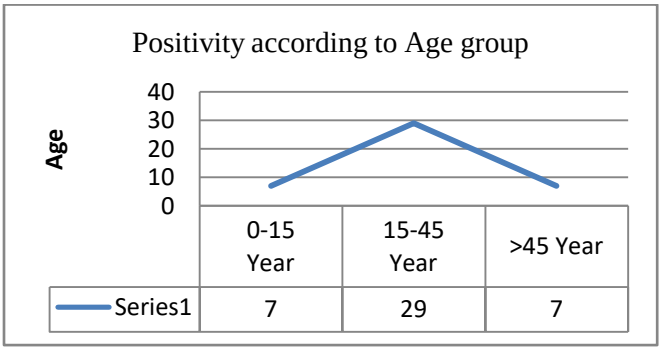

Figure 6: EPTB Positivity rate according to age group

Sample	Total	Positive	%
Pus	67	27	40%
Lymph node	19	7	37%
BAL	4	3	75%
Tissue & Biopsy	27	4	15%
Fluid	2	2	100%

Table 1: Detection of EPTB from Sample

Conclusions

Diagnosis of EPTB poses a major challenge to health care facilities and the research community in resource constrained settings because of paucibacillary samples, which reduces the sensitivity to conventional diagnostic tests [2,4].The advent of molecular test such as GeneXpert MTB/RIF seems to bring a considerable gain in the diagnosis of Extrapulmonary tuberculosis and rifampicin resistance directly from clinical specimens, especially in the case of paucibacillary sample as phenotypic methods are more time consuming, therefore shorten the turnaround time (2 h) [2,3,4].It is more accurate test to diagnose various forms of EPTB, which can easily be incorporated in the routine TB control programme, would contribute significantly towards improving EPTB case-detection and thus reducing the morbidity and mortality [4].Conversely, the adoption of molecular diagnostic tests for rifampicin resistance makes it possible to reduce the time-to-results and additively increases the diagnostic sensitivity; clinicians could rapidly implement the anti-tuberculosis drug without having to wait for the results of the culture [2].Early diagnosis and early initiating of anti-tubercular treatment plays a very crucial role in control and spread of the disease and further limits the mortality and morbidity of disease [11].

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Abbreviations

TB – Tuberculosis

EPTB - Extrapulmonary Tuberculosis

WHO – World Health Organization

MTB - Mycobacterium Tuberculosis Bacilli

M.tb – Mycobacterium Tuberculosis

RIF – Rifampicin

BAL – Bronchoalveolar Lavage

BCE – Before Common Era

CBNAAT - Cartridge-based nucleic acid amplification test

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