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Diagnosis of Extra Pulmonary Tuberculosis By Using Xpert® MTB/RIF Assay (CBNAAT) And MGIT Liquid Culture.

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Abstract

Background: *Globally, India Is A Home For More Than 25% Of Global Tuberculosis (TB) Burden. The Sensitivity Of Smear Microscopy And Its Inability To Detect Drug Resistance Limits Its Impact On TB Control. Early And Accurate Diagnosis Is The First Critical Step In Controlling TB. The Control Of TB Is Hampered By Diagnostic Methods With Sub-Optimal Sensitivity, Particularly For The Detection Of Drug Resistant Forms And In Patients With Human Immunodeficiency Virus (HIV) Infection. Early Detection Is Essential To Interrupt Transmission And Reduce The Death Rate, But The Complexity And Infrastructure Needs Sensitive Methods Which Limit Their Accessibility And Effect. Diagnosis Of Extra Pulmonary TB (EPTB) Remains Especially Challenging Since The Number Of Mycobacterium Tuberculosis (MTB) Bacilli Present In Tissues At Sites Of Disease Is Often Low And Clinical Specimens From Deep-Seated Organs May Be Difficult To Obtain. Histology Is Time-Consuming To Undertake And Establishing A Diagnosis Of TB With High Specificity Remains Difficult. Tissue Microscopy After Special Staining Is Often Negative And When Mycobacteria Are Seen, It Is Impossible To Distinguish MTB From Nontuberculous Mycobacterial Disease. The Xpert® MTB/RIF Assay (Cepheid Inc., CA, USA) Marks An Important Development In The Field Of Rapid Molecular TB Diagnostics Keeping This View The Study Was Under Taken To Diagnose Extra Pulmonary Tuberculosis In Patients Admitted In Govt General Hospital.*

Aim And Objectives:

1 To Diagnose Extra Pulmonary Tuberculosis By Gene Xpert®, Florescent Microscopy And Liquid Cultures
2. To Evaluate The Sensitivity and Specificity Of Gene Xpert® In Extra Pulmonary Tuberculosis And Compared With LED Fluorescent Microscopy And Liquid Culture MGIT960.
3 Liquid Cultures Were Taken As Gold Standard In Our Study.

Methods: *A Descriptive Study Was Conducted At Government General And Chest Hospital, Visakhapatnam AP, India During 2018 Jan To Aug 2018. The Study Population Included All The Extra Pulmonary Presumptive TB Cases Who Were Subjected For Further Investigations By* ***CBNAAT, Fluorescent Microscopy*** *And* ***Liquid Culture****.*

Results: *Of The 103 Extra Pulmonary Samples, The Sensitivity And Specificity By CBNAAT Was 80.8% And 87.5% Respectively; While That For Fluorescent Microscopy Was 36.1% And 98% Respectively, When Compared With Liquid Culture Which Is The Gold Standard In Our Study. Out Of The 103 Samples CBNAAT Was Positive In 45 Of Which 3 Were Rif Resistant. Compare With Culture Result CBNAAT Had Sensitivity And Specificity Of 91.6% For FNAC Samples.*

Conclusion: *In Our Study Gene Xpert® Had A Sensitivity Of 80.8% And Specificity Of 87.5 In Extra Pulmonary Samples. In Addition Gene Xpert® Has Detected 3 Cases Of Rif Resistance Which Is Not Possible With Florescent Microscopy. It Has A Notable Advantage Of Detecting TB Within Two Hours Which Is Acceptable To All Clinicians To Institute Early Treatment. CBNAAT Is One Of The Rapid Diagnostic Tests Available In The Country And It Should Be Routinely Used Under The Public And Private Health Sector Effectively To Detect Early, Tuberculosis In Extra Pulmonary Samples.*

Key Words: *Cartridge-Based Nucleic Acid Amplification Test (CBNAAT); Tuberculosis;*
Fluorescent Microscopy; MGIT: MYCOBACTERIAL GROWTH INDECATOR TUBE MDR-TB: Multi-Drug Resistant TB;

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I. Introduction

TB remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assays(1) India has the highest number of Tuberculosis (TB) cases in the world, with over two million TB cases every year. Annually, one fourth of the global incident TB cases occur in India. Early and accurate diagnosis is the first critical step in controlling TB. The control of TB is hampered by diagnostic methods with sub-optimal sensitivity, particularly for the detection of drug resistant forms and in patients with human immunodeficiency virus (HIV) infection. Early detection is essential to interrupt transmission and reduce the death rate, but the complexity and infrastructure needs sensitive methods which limit their accessibility and effect.(2).

Diagnosis of extra pulmonary TB (EPTB) remains especially challenging since the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. Histology is time-consuming to undertake and establishing a diagnosis of TB with high specificity remains difficult. Tissue microscopy after special staining is often negative and when mycobacteria are seen, it is impossible to distinguish MTB from nontuberculous mycobacterial disease. Reliance on culture, the main stay of diagnosis, often leads to considerable delays, compromising patient care and outcomes.

Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. The US CDC recommends that nucleic acid amplification tests be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB [3]. However, no recommendation exists for their use in the investigation of patients suspected of having EPTB as the evidence base is limited.

The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics [4,5]. This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h. Since Xpert MTB/RIF was specifically developed and optimized for testing sputum samples and initial large-scale evaluations were in patients with pulmonary TB, WHO endorsement specifically applied to the investigation of pulmonary TB. More recently, however, evaluations of the assay have extended to a variety of non respiratory clinical samples from patients with EPTB. The evidence base for use in the investigation of EPTB remains comparatively weak, however, and many more studies assessing a variety of clinical samples other than sputum are therefore needed. However, compared with pulmonary disease, investigation for use in EPTB is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analyses and in extraction of MTB DNA from samples, the challenge of providing a rigorous gold standard for comparison, and the range of potential ways of processing samples prior to analysis.

Aim and objectives:

1 To diagnose extra pulmonary tuberculosis by gene Xpert®, florescent microscopy and liquid cultures

2. To evaluate the sensitivity and specificity of gene Xpert® in extra pulmonary tuberculosis and compared with LED Florescent microscopy and liquid culture MGIT960.

3 liquid cultures were taken as gold standard in our study.

Inclusion criteria:

1 all clinically suspected cases of extra pulmonary TB.

Exclusion criteria:

1 Samples received without clinical history.

2 samples without request for all three tests.

3 patients with history of lung malignancy or fungal infection.

4 patients with previous history of extra pulmonary TB or on treatment.

II. Material and Methods

A study was conducted at Government General and Chest Hospital, Visakhapatnam AP, India during 2018 Jan to Aug 2018(8 months) and the study population included all the extra pulmonary presumptive TB cases who were subjected for further investigations by CBNAAT, Fluorescent Microscopy and liquid culture.

Sample Collection from Extra Pulmonary Sites: The samples were collected under sterile aseptic conditions depending on the site of infections in sterile container.

FNAC from lymphnodes, (1 to 2 ml) CSF from suspected TB meningitis (2 to 3 ml)

pleural fluid from suspected TB of lung(2 to 5 ml) BAL fluid bronchial lavage (2 to 5 ml)

ascitic fluid, synovial fluid(2 to 5 ml) were collected and transported.

Transport of sample: extra pulmonary specimens were transported in cool boxes which maintain temperatures below 20C for specimens to be compatible for liquid culture systems as well as molecular methods. Triple packing system was utilized for transportation.

Processing of samples: Processing of extra pulmonary samples for MGIT960 requires the final inoculum to be in an ideal condition that will not interfere with the fluorescence.

Pus and other muco-purulent specimens. Thick pus of volume >10 ml is decontaminated using the NALC – NaOH method as for sputum

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Bronchial washings. Processed using NALC-NaOH like sputum

Tissue. The tissue was homogenized in a tissue grinder with a small quantity of sterile saline or water (2-4 ml). the homogenized specimen was decontaminated using NALC-NaOH procedure as in sputum. Resuspend the sediment with phosphate buffer. If the tissue grinder was not available, a mortar and pestle was used. Tissue was also placed in a Petri dish with sterile water (2-4 ml) and torn apart with the help of two sterile needles.

Other body fluids (CSF, synovial fluid FNAC fluid and pleural fluid) As these fluids are collected usually under aseptic conditions, they required only milder decontamination If the specimen volume was more than 10 ml, concentrate by centrifugation at about 3000x g for 15-20 minutes was done Liquefy thick or mucoid specimens prior to centrifugation by adding NALC powder (50-100 mg). Resuspend the sediment in about 5 ml of saline. Decontamination was done by NALC-NaOH procedure.

All samples were divided in to three portions and subjected to Fluorescent Microscopy, CBNAAT, and Digestion and decontamination for liquid culture. The smears were prepared by the auramine-rhodamine acid-fast staining method as per the RNTCP stander guidance

For CBNAAT examination the sample reagent were added at a 2:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge.

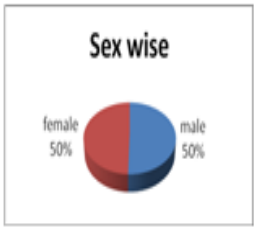
The decontaminated specimens were inoculated into MGIT liquid culture medium for growth detection. The smear positive specimens were evaluated within two weeks at the latest, while the smear-negative specimens were studied immediately after the growth of culture.

III. Results

A total of 103 specimens were collected during the study period. Amongst the samples received 52 (50.4%) were males and females were 51 (49.6%) Table 1

Table no 1 Distribution of EP samples based on sex

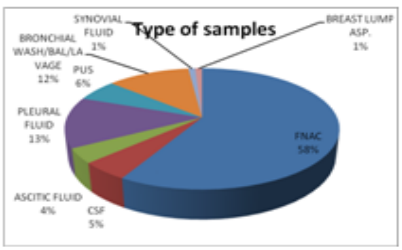
Sex	Nos
Male	52
Female	51
Total	103



out of the total 103 samples that were examined the majority were from FNAC lymphnode 60(58%),followed by plerural fluid 14(13.5%),bronchial fluids 12(11.6%),and the rest of extra pulmonary samples 16.5%(pus 5.8%,CSF 4.8%,ascitic fluid 4% synovial fluid 1%,breast lump aspirate1%) Table2.

Table no 2

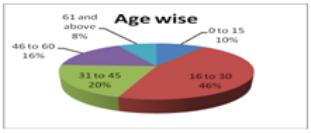
Distribution of EP samples based on type	
type of sample	no of samples
FNAC	60
CSF	5
ASCITIC FLUID	4
PLEURAL FLUID	14
PUS	6
BRONCHIAL WASH/BAL/LAVAGE	12
SYNOVIAL FLUID	1
BREAST LUMP ASP.	1
Total	103



Majority of the samples 47 were in the age group of 16 to 30 years (45.6%),followed by 21 in the age group of 31to 45 (20.3%),17 (16.5%)in the age group of 46 to 60 years, and 10(9.7%) cases less than 15 years. In the age group of above 61 years was 8 samples (7.7%) Table 3

Table no 3

,Distribution of EP samples based on age	
Age	no of samples
0 to 15	10
16 to 30	47
31 to 45	21
46 to 60	17
61 and above	8
Total	103



The comparison of CBNAAT and smear by fluorescent microscopy and liquid culture shown in Table4.

Table no 4

,Distribution of EP samples CBNAAT ,Liquid culture and Fluorescent microscopy				
type of sample	no of samples	CBNAAT Positive	FM positive	culture positive
FNAC	60	25	10	24
CSF	5	2	0	1
ASCITIC FLUID	4	1	0	1
PLEURAL FLUID	14	10	3	10
PUS	6	1	1	2
BRONCHIAL WASH/BAL/LAVAGE	12	6	3	7
SYNOVIAL FLUID	1	0	0	1
BREAST LUMP ASP.	1	0	0	1
Total	103	45	17	47

Out of the total 103 samples tested CBNAAT was positive in 45 (43.6%). and out of these liquid culture was also positive in 38.Out of the 58 CBNAAT negatives, 9were positive for liquid culture which shows sensitivity and specificity of CBNAAT 80.8% and 87.5% respectively. This has correlated with Tortoli and colleagues (table 6)

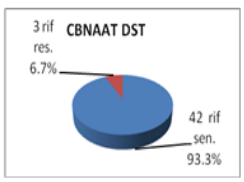
The majority of the extra pulmonary samples were FNAC of lymph nodes which indicates the high prevalence of primary tuberculosis in our study. Out of the 60 FNAC samples received 25 were positive by CBNAAT and 22 by liquid culture and only 10 were positive by Fluorescent Microscopy. Compared with culture result CBNAAT had sensitivity and specificity of 91.6% respectively for FNAC. This has correlated with Laura maynard smith natasa larke et.al who had reported 96% and 93% sensitivity and specificity respectively For Pleural fluid samples Fluorescent Microscopy had detected of 3 out of 14(21.4%) where as CBNAAT detected 10 out of 14 (71.4%) and liquid cultures had detected 10 out of 14 (71.4%). The sensitivity and specificity compare to the gold slandered was 80% and 50% respectively

For all EP exudative fluids (Bronchial wash/Bal/Lavage,Synovial fluid,Pus,CSF,Ascitic fluid and Breast lump aspirate) the total samples received were 29 out of which Fluorescent Microscopy was positive in 4(13.7%), CBNAAT had detected 10(34.4%) and the liquid culture was positive in 13(44.8%). Out of the 25 smear negative samples, CBNAAT has detected 6(24%), because of pausi bacillary nature of the sample.

Out of the 45 CBNAAT positives rif sensitive was 42(93.3%) and 3(6.7%) were rif resistance. The rif resistance was seen in Pus,CSF,and pleural fluid table 5.

Table no 5

Distribution of EP samples CBNAAT Rif Sensitive and resistance			
type of sample	CBNAAT POSITIVE	RIF SEN	RIF RES
FNAC	25	25	0
CSF	2	1	1
ASCITIC FLUID	1	1	
PLEURAL FLUID	8	7	1
PUS	2	1	1
BRONCHIAL WASH/BAL/LAVAGE	7	7	0
SYNOVIAL FLUID	0	0	0
BREAST LUMP ASP.	0	0	0
Total	45	42	3



Sensitivity In our study was 80.8% which correlate with Laura maynard smith natasa larke et.al, Tortoli and colleagues, Stephen dlawn and colleagues. The specificity in our study 87.5% was less then the other studies which could be due to the short sample size and duration of study table no 6.

Table no 6 Comparative study of EPTB

Study	sensitivity	specificity
Laura maynard smith natasa larke et.al.	83%	98%
Tortoli and colleagues	81.3%	99%
Stephen dlawn and colleagues	79%	97.3%
Suresh ramana et.al our study	80.8%	87.5%

IV. Discussion

TB remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assays(1) Diagnosis of extra pulmonary TB (EPTB) remains especially challenging since the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. The US CDC recommends that nucleic acid amplification tests be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB [3] The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics [4,5]. More recently, however, evaluations of the assay have extended to a variety of non respiratory clinical samples from patients with EPTB. This study was under taken to diagnose extra pulmonary tuberculosis by **gene Xpert®, florescent microscopy and liquid cultures** in our centre and to evaluate the sensitivity, specificity of gene Xpert® in extra pulmonary tuberculosis and compared with LED Florescent microscopy and liquid culture MGIT960.

The liquid cultures were taken as gold standard in our study.

A total of 103 specimens were collected during the study period. Amongst the samples received 52 (50.4%) were males and females were 51 (49.6%) Table 1. Out of the total 103 samples that were examined the majority were from FNAC lymphnode 60(58%),followed by plerural fluid 14(13.5%),bronchial fluids 12(11.6%),and the rest of extra pulmonary samples 16.5%(pus 5.8%,CSF 4.8%,ascitic fluid 4% synovial fluid 1%,breast lump aspirate1%) Table2. Majority of the samples 47 were in the age group of 16 to 30 years (45.6%), which indicates the high prevalence of primary tuberculosis. This was followed by 21 in the age group of 31to 45 (20.3%),17 (16.5%)in the age group of 46 to 60 years, and 10(9.7%) cases less than 15 years.(7.7%) of the samples belong to above 61 years Table 3.

In the present study 103 samples were included the sensitivity of CBNAAT for extra pulmonary samples was 80.8% and FM smear which was 36.1%.when compared with liquid cultures gold standard The observed sensitivity of Xpert MTB RIF 80.8% is consistent with other studies Laura maynard smith natasa larke et.al, Tortoli and colleagues,and Stephen dlawn and colleagues table 6.

Among individual extra pulmonary samples, the sensitivity of CBNAAT was highest among lymph nodes 91.6% when compared to Fluorescent Microscopy 41.6%. Inclusion of CBNAAT in the initial diagnosis of tubercular lymphadenopathy in addition to the FNAC would decrease the over diagnosis of tuberculosis and injudicious use of anti-tuberculosis treatment (ATT).

For Pleural fluid samples Fluorescent Microscopy had detected of 3 out of 14(21.4%) where as CBNAAT detected 10 out of 14 (71.4%) and liquid cultures had detected 10 out of 14 (71.4%). The sensitivity and specificity compared to the gold standard was 80% and 50% respectively.

For all EP exudative fluids (Bronchial wash/Bal/Lavage,Synovial fluid,Pus,CSF,Ascitic fluid and Breast lump aspirate) the total samples received were 29 out of which Fluorescent Microscopy was positive in 4(13.7%), CBNAAT had detected 10(34.4%) and the liquid culture was positive in 13(44.8%). Out of the 25 smear negative samples, CBNAAT has detected 6(24%), because of pausi bacillary nature of the sample. Out of the 45 CBNAAT positives rif sensitive was 42(93.3%) and 3(6.7%) were rif resistance. The rif resistance was seen in Pus,CSF,and pleural fluid table 5. This suggests that CBNAAT is a sensitive tool to detect TB in smear negative extra pulmonary tuberculosis, and rifampicin resistance.

Our study findings suggest that CBNAAT has higher sensitivity for detection of extra pulmonary tuberculosis cases compared to Fluorescent Microscopy. The WHO has also recommended the CBNAAT for routine use under programmatic conditions for extra pulmonary tuberculosis.

The study has following limitations (a) it was an experimental study and it did not adapt a rigorous study design while the samples included were small to make a generalized statements (b) the culture is known to be a suboptimal standard for extra pulmonary TB and the same was used as standard to compare with CBNAAT and sputum smear.

To conclude:

In our study **CBNAAT** detected 28 out of the 86 smear negatives by fluorescent microscopy.

In FNAC samples **CBNAAT** detected 15 out of the 50 smear negatives, which would have been reported as negative. This underlines the importance of **CBNAAT** over fluorescent microscopy in diagnosing tuberculosis in extra pulmonary samples. CBNAAT is one of the rapid diagnostic tests available in the country and it should be routinely used under the public and private health sectors efficiently to detect extra pulmonary Tuberculosis.

Conflicts Of Interest

The authors declare that they have no conflicts of interest.

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