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RAPID DETECTION OF MYCOBACTERIUM TUBERCULOSIS AND RIFAMPICIN RESISTANCE IN EXTRAPULMONARY SAMPLES USING GENE XPERT MTB & RIF ASSAY.

Medical Microbiology

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ABSTRACT

Background: About one-quarter of World's population has latent TB. Still exact magnitude of EPTB infection is not known. Diagnosis of EPTB remains responsibility challenging since the number of MTB bacilli present in tissues at sites of diseases is often low and clinical specimens from deep seated organs may be difficult to obtain.

Material and Method: Total of 101 specimens were collected as per as the RNTCP guidelines from patients suspected of Extrapulmonary Tuberculosis. Sample was divided into two parts, first part was used for fluorescent microscopic smear and second part is used for processing on Gene X pert.

Results: Out of total 101 specimens n=26,(25.74%) were GeneXpert positive. All the specimens positive by fluorescent microscopy were also positive by GeneXpert ® MTB/RIF assay. The number of cases detected by Xpert ® MTB/RIF assay were significantly higher ($p<0.01$).

Conclusion: GeneXpert method is more sensitive in detection of Mycobacterium tuberculosis in Extrapulmonary tuberculosis than fluorescent microscopy technology. Additionally it also guide in proper treatment of patient by simultaneously detection of rifampicin sensitivity pattern.

KEYWORDS

Extrapulmonary tuberculosis, fluorescent microscopic, GeneXpert.

INTRODUCTION

TB remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assay.[1] About one- quarter of World's population has latent TB. Among the total tuberculosis cases, EPTB is now beginning to emerge from the shadows of its own senior Cousins Pulmonary Tuberculosis. It is a protean disease, which can visually affect all organs of the body. The most common site affected is lymph node followed by pleura, GIT, bones and joints, meninges and peritoneum.[2]

Though Pulmonary Tuberculosis is very much rampant throughout all ages, the EPTB infections are on increase. This increased incidence might be due to more awareness of the patients and the physicians. But still exact magnitude of EPTB infection is not known. Crux of the problem remains in having authentic diagnostic criteria and once this is achieved exact estimate of the problem can be carried out. Pulmonary Tuberculosis, being highly infectious, gets maximum attention. EPTB though non - infectious lead to considerable suffering and disability and certainly merits more attention. Diagnosis of EPTB remains responsibility challenging since the number of MTB bacilli present in tissues at sites of diseases is often low and clinical specimens from deep seated organs may be difficult to obtain.[3]

Among the various method PCR is one of them. It is an in- vitro method of amplification. Presence of conserved region of the Mycobacterial DNA in clinical specimen like pleural fluid, cerebrospinal fluid, ascitic fluid and lymph node biopsy can be amplified and detected by this method(9). Nucleic acid amplification technique due it's rapidity and sensitivity not only help in early diagnosis and management of tuberculosis but also curtail the transmission of disease.[3] The Gene Xpert MTB/RIF is a cartridge based nucleic acid amplification test for rapid tuberculosis diagnosis and rapid antibiotic sensitivity test simultaneously. It is an automated diagnostic test developed by laboratory of Professor David Alland at the University of Medicine and Dentistry of New Jersey, (UMDNJ).[4] Cepheid Inc and foundation for innovative New Diagnostics, with additional financial support from US national Institute of Health(NIH). In December 2010, WHO endrossed the Gene Xpert MTB/RIF for use in TB Endemic countries(5).This test have the potential to improve the diagnosis of TB in those that are likely to missed by traditional tests.[6],[7]

TB remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assay.[8] Anti Tuberculosis (TB) drug resistance is a major public health problem that threatens the progress made in TB care and control World wide.[9] So the present study aims to find the performance of Gene Xpert (automated RTPCR) for detection of Mycobacterium Tuberculosis in clinically suspected EPTB and comparing it with fluorescent microscopy. This study also aims to find out the drug resistance pattern of Mycobacterium for Rifampicin.

AIMS

1. To study frequency of EPTB in Tertiary Care Hospital.
2. To Evaluation the performance of Gene Xpert for detection of Mycobacterium Tuberculosis in clinically suspected cases.
3. To study drug resistance pattern of Mycobacterium for Rifampicin.

INCLUSION

Clinically suspected cases of Extrapulmonary Tuberculosis.

EXCLUSION.

1. Patients on Anti-Tuberculosis treatment for more than 1 month.
2. Patient age <12 years age.

MATERIALAND METHODS

The Present study was conducted to find out the performance of GeneXpert for detection of Mycobacterium tuberculosis in 101 clinically suspected cases of Extra pulmonary tuberculosis over a period of 18 months from December 2018 to May 2020. 101 specimens from clinically suspected cases of Extapulmonary tuberculosis were included on the basis of the following clinical criteria.

- Pyrexia of unknown origin
- No response to routine antibiotics.
- Radiological evidence suggestion of tuberculosis.

Patients name, age and sex, address, history, clinical findings and relevant investigations was recorded. Samples were collected before starting anti-tuberculosis treatment to the patient. Total of 101 specimens were collected as per as the RNTCP guidelines from patients suspected of Extrapulmonary Tuberculosis.

Pleural and ascitic fluid: Approximately 10 ml of aspirated fluid samples was collected in a sterile falcon tube by doing plural and ascitic tapping under all aseptic precautions.

Cerebrospinal fluid (CSF): 3 to 5 ml of CSF was collected in a sterile bulb by doing lumbar puncture under all aseptic precautions.

FNAB (Fine Needle Aspiration Biopsy): Fine needle aspiration biopsy sample from lymph node was aspirated by 25 No. gauge needle and disposable syringe under all aseptic precautions.

Biopsy sample : Biopsy sample from tuberculoma breast was collected in sterile bulb containing normal saline and formalin separately.

Pus sample: Pus sample from cold abscess was aspirated with disposable syringe and needle.

Sample was divided into two parts, first part was used for fluorescent microscopic smear and second part is used for processing on Gene X pert. The entire procedure of both specimens was carried out in a Biosafety Cabinet Class II with level 2 Biosafety practices. First part of sample was subjected to fluorescent staining was done using Auramine O stain as per the RNTCP guidelines.[10] Second part of the same sample is subjected to Gene X pert MTB/RIF assay testing was done according to manufactures Instructions.[11]

OBSERVATION AND RESULT

Table 1: Total specimen wise distribution of clinically suspected Extrapulmonary Tuberculosis cases.

Clinical specimen	No. of cases(%)
Pleural fluid	36 (35.64)
LN Aspirate	34 (33.66)
CSF	12 (11.88)
Pus	7 (6.93)
Gastric lavage	5 (4.95)
Ascitic fluid	3 (2.97)
FNAB	3 (2.97)
Peritoneal fluid	1 (2.97)
Total (N)	**101**

In this study maximum number of cases are patients with Pleural Effusion n=36, (35.64%), which is statistically significant (chi square= 111.99, Df = 7, P<0.01) followed by cases with lymph node aspirate n=34, (33.66%).

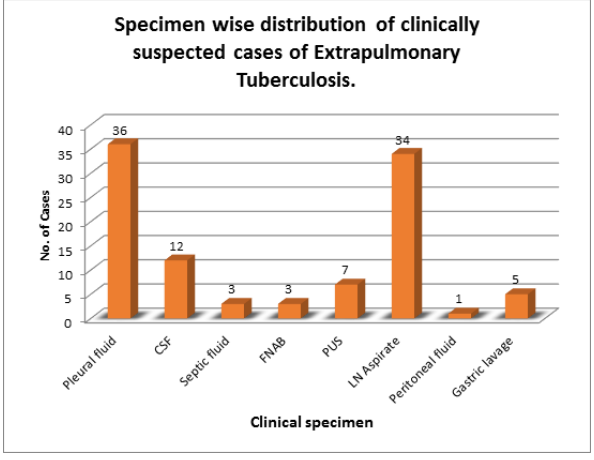


Table2: Showing fluroscent microscopy smear results in total number of EPTB cases.

Fluroscent microscopy	Result
Positive	10 (9.90%)
Negative	91 (90.09%)
Total (n)	**101 (100%)**

Out of total 101 clinically suspected cases n= 10, (9.90%) cases revealed bacilli on fluorescent microscopy, and n= 91, (90.09%) were negative on fluorescent microscopy.

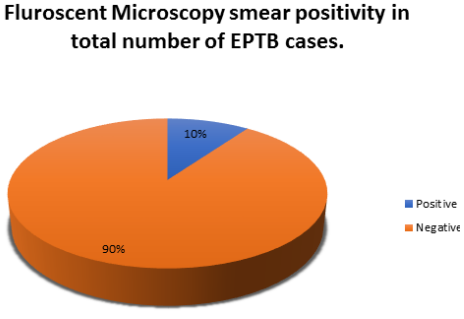


Table 3: Showing GeneXpert results among Extrapulmonary cases.

GeneXpert Result	Number of specimen
MTB Detected	26 (25.74%)
MTB Not Detected	75(74.16%)
Total (n)	101(100%)

Out of total 101 clinically suspected EPTB cases, 101 different clinical speciemn were subjected to GeneXpert for detection of MTB , n= 26 (25.74%) specimen revealed presence of MTB, while in n=75 (74.16%) specimen, MTB was not detected.

Table 4: Gender wise distribution of EPTB cases in which MTB was detected by GeneXpert.

Age group	Male	Female
12-19	1	1
20-29	5	9
30-39	1	4
40-49	4	0
50-59	1	0
60-69	0	0
>70	0	0
Total (n=26)	**12 (46.15%)**	**14 (53.85%)**

Among 26 clinically suspected cases of EPTB in which MTB was detected n=12, (46.15%) were male and n=14, (53.85%) were female.So the ratio Male: Female = 1:1.17. No statistically significant difference between the male and female is seen (Z=1.10, P>0.05)

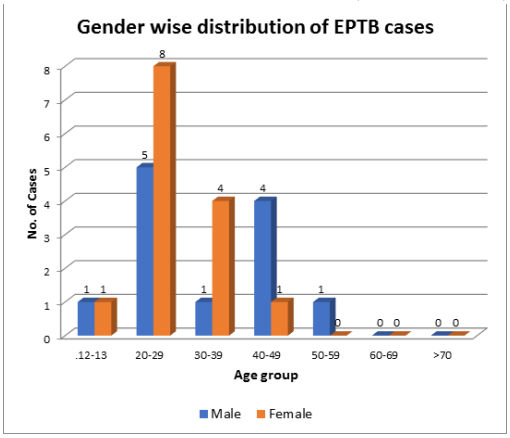


Table 5: Comparision of GeneXpert with Fluorscent Microscopy

Result	Genexpert	Fluorscent Microscopy
Positive	26	10
Negative	75	91
Total	**101**	**101**

Out of 101 clinically suspected cases in n=26 (25.74%) clinical specimen MTB were detected. Ten specimens revealed MTB by fluorescent microscopy and 16 specimens were negative on fluorscent microscopy.The difference was statistically significant (P=<0.01, Z=2.94).

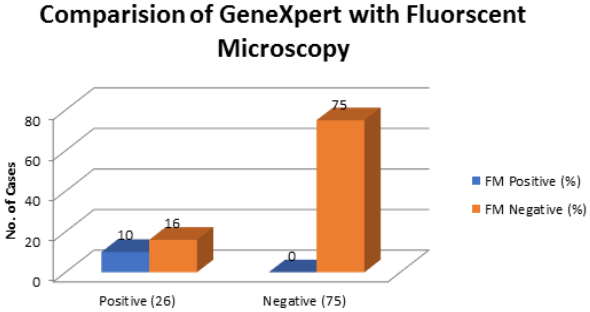


Table 6: Showing Bacterial load Detected by GeneXpert among total number of suspected EPTB cases.

Bacterial load	No. of cases detected on GeneXpert (%)
High MTB Detected	1 (3.85%)
Medium MTB Detected	4 (15.38)
Low MTB Detected	9 (34.61)
Very Low MTB Detected	12 (46.15)
Total	**26 (100%)**

Maximum specimen had very low bacterial load n=12, (6.15%) and low bacterial load n=9, (34.61%) on GeneXpert Assay. Only one

sample (Pleural fluid) had high Bacterial load detected.

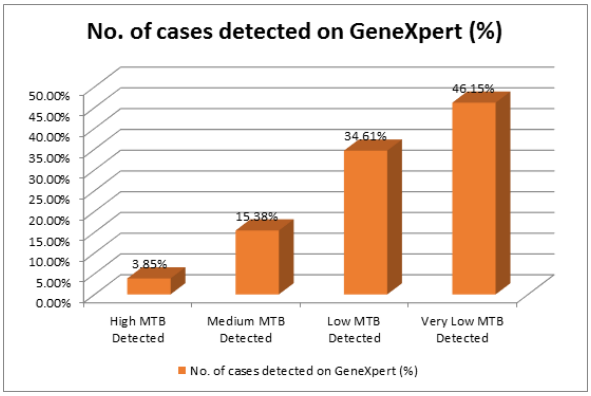
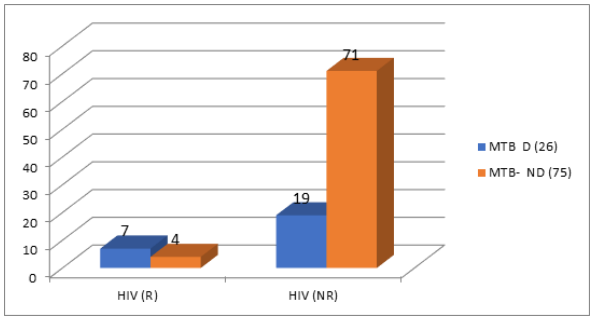


Table 7: HIV Status of patients suspected of Extrapulmonary tuberculosis.

Cases	HIV (reactive)	HIV (Not reactive)
MTB D (26)	7 (26.92%)	19 (73.07%)
MTB- ND (75)	4 (5.33%)	71 (94%)
Total (101)	11 (10.89%)	90 (89.11%)

In the above table, 26.92% (7 cases) were HIV Reactive and 73.07 % (19 cases) were HIV non Reactive. According to statistical analysis (chi square = 9.27, Df = 1, P < 0.01) the above data shows significant association. Among the 101 cases 11 cases were HIV reactive and among these n= 7 (26.92%) cases MTB was detected, while in n=4 (5.33%) cases MTB not detected.



DISCUSSION

The present study is on performance of Gene Xpert ® MTB/RIF assay, a cartridge based nucleic acid amplification test and LED fluorescent microscopy in Extrapulmonary Tuberculosis, has provided expected findings. Xpert ® MTB/RIF assay detected greater number of cases than LED fluorescent microscopy performed better than acid fast stained light microscopy. The need for a more accurate test in settings of EPTB to provide confirmatory evidence of clinical and radiological suspicion has long been felt. Xpert ® MTB/RIF assay also provided rapid information on rifampicin sensitivity, there by playing a dual and significant role.

In the present study the most frequently reported clinical form of EPTB was (table:1)Pleural effusion n=36, (35.64%) followed by lymph node aspirate n=34, (33.66%). Similar findings have been reported by *Praksha SR et al* [12] (28.03%, Karnataka, India, 2013), *Mustafa Kursat Ozvaran et al* [13] (66%,turkey, 2007) and *Sanjivani J. Keny etal* [14] (38.67% Goa, 2017) where Pleural TB was the commonest type of EPTB. Pleural tuberculosis is thought to be due to delayed hypersensitivity reaction to Mycobacterial antigens in the pleural space[15]. When subpleural foci of Mycobacteria rupture, bacilli gain access into the pleural space .[16,17,18,19] accounting for its higher frequency in EPTB.

Lymph Node TB has also been reported as the most frequent form of EPTB.[20,21,22,23] in many studies. In India and other developing countries, lymph node tuberculosis continues to be the most common form of EPTB.[21] It is caused due to the spread of infection from primary site to the draining lymph-node through lymph-haematogenous route .[24]

Some authors considered pleural TB as an early manifestation of primary Mycobacterial infection.[25,26]

As shown in the table 2:55.45% were male and 44.55% were female. According to statistical analysis (Z = 1.09, P > 0.05) there is no significant difference in gender wise distribution of suspected cases Extrapulmonary tuberculosis.

EPTB was found to be higher in the male than female, which is similar to the study done by *Shrivastava AK et all (*2015).[27] Male preponderance has also been found in various other studies from India and other countries like Ethopia, Nigeria, Turkey etc.[28,29,30] This could be due social stigma associated with TB which hinders female population to seek medical assistance from DOTS centre in developing countries.[31]

As shown in the table 3: Maximum number of EPTB positive case (53.85%) were in the age range of 20-29 years in the present study. EPTB was most prevalent among young adults in the present study, with a decline in the older age group. This may be due to the more active lifestyle of younger age group and more time spent out, causing increased risk of exposure. Exposure to Mycobacterium Tuberculosis occurs at an early age in India and following infection there is a 23.7% risk of developing EPTB at 5 years of contact[32]

Out of total 101 specimens n=26, (25.74%) were GeneXpert positive. GeneXpert detected a additional of 16 cases in comparison to fluorescent microscopy. Comparison of GeneXpert ® MTB/RIF assay with fluorescent Microscopy. All the specimens positive by fluorescent microscopy were also positive by GeneXpert ® MTB/RIF assay. The number of cases detected by Xpert ® MTB/RIF assay were significantly higher ($p<0.01$) and correlates with the positivity reported in literature.[33,34,35]

All samples which were FM smear-positive were also GeneXpert MTB/RIF assay positive. This is due to the high sensitivity (97.4%) of Gene Xpert MTB/RIF assay in smear-positive samples across all sample types.[36] Bates et al. in their study reported a better detection rate with Gene Xpert MTB/RIF assay when compared to smear microscopy.[37]

As shown in table 6, Low and Very Low Load was seen in 80.76% of the specimens tested positive on Xpert ® MTB/RIF assay. Similar findings were reported by *Enrico Tortoli et al* in 2012 [37] where 78.4 % of specimens showed Low and Very Low Load. This result correlates with the fact that EPTB is a paucibacillary disease.[14]

As shown in table 7: 26.72% (7 cases) were HIV positive and 73.07% (19 cases) were HIV negative among extrapumonary positive cases. As per RNTCP , the prevalence of EPTB in non –HIV patients was 15-20%.[39] In our study it was 73.07%, which is more than the RNTCP statistics. This is similar to study done by *Shrivastava Ak et all* (2015) [40] showing 26.47% which is more than RNTCP statistics. This increase in prevalence is due to availability of advanced diagnostic facilities at low cost in medical college setting and tertiary care referral centre.[29]

CONCLUSION

In present study the frequency of extrapulmonary tuberculosis cases in tertiary care hospital is 25.74%. Amongst the fluorescent staining and GeneXpert cartridge based automated system,GeneXpert detected more number of cases which is statistically significant. Detection of Mycobacteria tuberculosis by culture method is time consuming and need to be performed by using biosaftey cabinet by strictly observing all biosafety guidelines.

Moreover in Extrapulmonary tuberculosis cases where clinical diagnosis is not easy, where disease present with atypical features, quick confirmatory results are required to start the treatment and get better outcome. That is why GeneXpert should be used. Occurrence of Rimapicin resistance is 3.84% among extrapulmonary tuberculosis cases indicates that drug resistance pattern is required for effective treatment.

Present study revealed that clinical suspicion of Extrapulmonary tuberculosis in 20-29 year age group cannot be ignored. GeneXpert method is equally more sensitive in detection of Mycobacterium tuberculosis in Extrapulmonary tuberculosis than fluorescent microscopy technology. Additionally it also guide in proper treatment of patient by simultaneously detection of rifampicin sensitivity pattern.

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