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GENE XPERT – A RAPID METHOD FOR DETECTION OF EXTRA PULMONARY TUBERCULOSIS AND RIFAMPICIN RESISTANCE IN SUSPECTED EXTRA-PULMONARY SAMPLES.

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ABSTRACT **Introduction** - Tuberculosis (TB) has been a major global public health problem from times immemorial. There are two clinical manifestations of tuberculosis (TB): pulmonary TB (PTB) and extra-pulmonary TB (EPTB). It is difficult to diagnose EPTB with the conventional methods. Gene Xpert has come up with the rapidity and simplicity for detection of mycobacterium bacilli and rifampicin resistance.

Aims-

1. To evaluate the role of Gene Xpert MTB/RIF Assay in diagnosing EPTB and assessment of rifampicin resistance.
2. To identify the tubercular bacilli in EPTB samples using staining techniques.
3. To isolate the tubercular bacilli by conventional culture method.

Methods- Total 375 extra-pulmonary samples were collected from suspected cases of EPTB, were subjected to Gene Xpert, culture on LJ media and staining techniques (ZN staining and fluorescent staining). **Results-** Of 375 samples subjected to Gene Xpert MTB/RIF Assay, MTB was detected in 75 (20%) cases, which included 44(58.67%) males and 31(41.33%) females. It simultaneously detected rifampicin resistance in total of 13(3.46%) cases. Maximum positivity by Gene Xpert was seen in lymph node tissues and aspirates (35.71%) followed by pus (32.88%).

Conclusion- Gene Xpert is excellent tool for diagnosis of EPTB.

KEYWORDS : Extra pulmonary tuberculosis, Gene Expert, rifampicin resistance

INTRODUCTION

Tuberculosis (TB) has been a major global public health problem from ancient times.[1] There are two clinical manifestation of tuberculosis (TB): pulmonary TB (PTB) and extra-pulmonary TB (EPTB). EPTB is significant health problem in both developing countries.[2] The major challenge in diagnosis is its atypical presentation which leads to delay in or deprivation of treatment .[3] Therefore high index of suspicion is necessary by a clinician to make early diagnosis. Diagnosis of EPTB is challenging as samples obtained from relatively inaccessible sites may be pauci-bacillary, collection of extra pulmonary samples is not easy from deep seated organs and amount of clinical sample may be inadequate.[4,5,6] In lower income countries this problem may be aggravated by lack of diagnostic infrastructure.[5]

Conventional methods like microscopic examination of acid-fast stain by ZN stain are very popular but they fail to provide required sensitivity and specificity for extra pulmonary samples.[4] Culture test have variable sensitivities (30%- 80%) and it very time consuming. Although culture the mainstay of diagnosis it compromises patients care and outcome due to considerable delays.[4,6,7]

Therefore, the WHO has endorsed the implementation of Gene Xpert MTB/RIF assay in December 2010.[6,8]The Xpert MTB/RIF (Cepheid Inc.) is an automated, user friendly and rapid test based on nested real time PCR assay and molecular beacon technology of MTB detection and RIF resistance. The results are obtained in short period of time (2 hours).[8]

AIMS AND OBJECTIVES

1. To evaluate the role of Gene Xpert MTB/RIF Assay in diagnosing EPTB and assessment of rifampicin resistance.
2. To identify the tubercular bacilli in EPTB samples using staining techniques.
3. To isolate the tubercular bacilli by conventional culture method.

MATERIAL AND METHODS

This cross-sectional study was carried out at IGGMC, Nagpur from December 2016 to May 2018. A total 375 EPTB samples were collected from suspected cases of EPTB. Suspected cases of tubercular pleural effusion, cold abscess, meningitis, lymphadenopathy, ascites, synovitis, endometrial fluid were included. Cases of isolated pulmonary TB (no extra-pulmonary component), pyogenic meningitis, non-tubercular pleural effusion, non-tubercular ascites, malignant & inflammatory and lymphadenopathy were excluded.

Statistical Analysis:

The significance of difference in proportion of males and females showing positive findings was evaluated using Z-test of proportion. Diagnostic evaluation carried out in terms of test positivity of ZN staining, fluorescent staining and LJ culture was compared with that of Gene Xpert MTB/RIF Assay using Z-test of proportion. Ethical clearance was obtained from the ethical committee of the Institute.

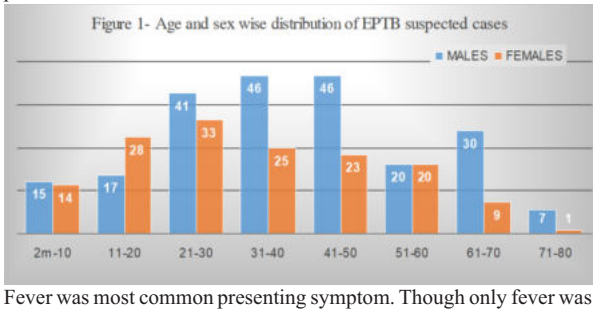
MATERIAL AND METHOD-

EPTB samples from each suspected extra-pulmonary case were taken. 1 sample was sent for Gene Xpert and other to microbiology lab. Septic samples were decontaminated and were used for staining and culture. Aseptic samples were directly used for staining (ZN stain and Florescent stain) and culture (LJ slopes).

OBSERVATION AND RESULTS-

A total of 375 suspected cases of extra-pulmonary tuberculosis were included in the study. Of them, 222 (59.2%) cases were males whereas 153(40.8%) were females. 23 (6.13%) patients out of 375 were HIV positive and were on ART.

Figure 1- Age and sex wise distribution of EPTB suspected cases



Fever was most common presenting symptom. Though only fever was present in 27 cases, it was associated with other symptoms related to system involved. Fever was followed by anorexia, cough, weight loss, lymph node swelling, breathlessness, abdominal fullness and abdominal pain etc. 46 patients had both fever and lymphadenopathy

while as 10 cases had fever, lymphadenopathy and weight loss. Most common site involved in our study was cervical lymph nodes 60.71% (34/56), followed by axillary (16.09%) and supraclavicular lymph nodes (8.92%),submandibular lymph nodes (5.35%), infra-clavicular lymph nodes (3.59%) and suprasternal lymph nodes (1.75%). 2 (3.59%) cases had multiple lymph node swellings. 6 females presented with the history of infertility.

Out of 375 extra-pulmonary samples included in this study, maximum were pleural fluid (38.13%) followed by pus (19.46%), ascitic fluid (16.26%), lymph node tissue and aspirates (14.93%), CSF (6.40%), gastric lavage (1.86%), endometrial tissues and aspirates (1.60%), synovial fluid (1.06%) and biopsy tissue (0.30%).

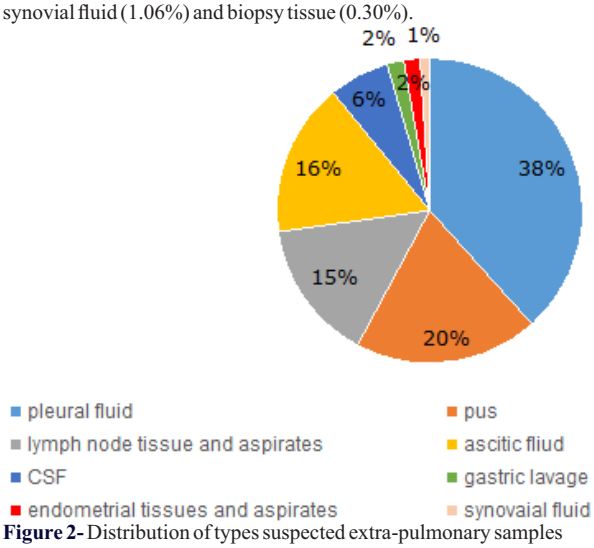


Figure 2- Distribution of types suspected extra-pulmonary samples

All 375 samples were subjected to Gene Xpert MTB/RIF Assay, of them MTB was detected in 75 (20%) cases, which included 44(58.67%) males and 31(41.33%) females. While MTB was not detected in 300 (80%) of EPTB cases. Rifampicin resistance was detected in 13(3.46%) cases. Of 222 males included in this study, Gene Xpert MTB/RIF Assay detected MTB in 44(19.81%) of males. Out of 153 females, 31(20.26%) were positive by this test.

Table 1 - Samples Wise Distribution By Gene Xpert (n= 375)

Samples	No. of samples	MTB detected (%)	MTB not detected (%)	Rifampicin resistance (%)
Pleural fluid	143	20(13.99)	123 (86.01)	04 (02.79)
Pus	73	24 (32.88)	49 (67.12)	05 (06.84)
Ascitic fluid	61	06 (09.83)	55 (90.16)	02 (03.27)
Lymph node tissues and aspirates	56	20 (35.71)	36 (64.29)	02 (03.57)
CSF	24	03 (12.50)	21 (87.50)	00
Gastric lavage	07	02 (28.57)	05 (71.43)	00
Endometrial tissues and aspirates	06	00 (00)	06 (100)	00
Synovial fluid	04	00 (00)	04 (100)	00
Biopsy tissue	01	00(00)	01(100)	00
Total	375	75 (20)	300 (80)	13 (03.46)

Maximum positivity of Gene Xpert was seen in lymph node tissues and aspirates i.e 35.71% (20 out of 56) followed by pus 32.88% (24 out of 73). Gene Xpert simultaneously detected rifampicin resistance in total of 13 cases. Maximum resistance was detected in 5 (6.84%) of 73 pus samples.

Table 2 – Gene Xpert Positive Result According To The Type Of EPTB Cases

Types of EPTB cases	No. of cases detected	MTB detected (%), n=75	Rifampicin resistance (%), n=13
New cases	306	44 (14.37)	3 (00.98)
Previously treated and cured	51	18 (35.29)	3 (05.88)
Treatment failure	08	05 (62.50)	2 (25.00)
Defaulter	10	08 (80.00)	5(50.00)
Total	375	75 (20)	13 (3.46)

Diabetes mellitus was most common associated morbid condition, followed by HIV, hypertension, hepatic failure and renal failure in suspected cases of EPTB. On Gene Xpert MTB/RIF Assay analysis 14(29.78%) out of 47 diabetes patients had associated EPTB and 2 (4.25%) had rifampicin resistance.13 (56.52%) of HIV reactive patients were co-infected with EPTB and rifampicin resistance was detected in 4(17.39%). 15 (15.62%) of 32 hypertensive patients and 1 (25%) patient of 4 hepatic failure had EPTB. MTB was not detected in 2 patients of renal failure.

Of total 375 samples, Gene Xpert detected maximum number of positive cases 75(20%) in suspected cases of EPTB. It was followed by fluorescent staining 43(11.47%), LJ culture 41(10.93%) and ZN staining 33(8.80%).

DISCUSSION

Total of 375 samples from suspected EPTB cases were included in this study. Our study showed that 44(19.81%) of 222 males and 31(20.26%) of 153 females were positive by Gene Xpert MTB/RIF Assay. A slightly higher proportion of females were positive for EPTB in our study, though it was not statistically significant.

Most common presenting feature in our study was fever. Prakasha R. et al (2012) in his study of clinical characteristics of types of TB, reported fever as most common symptom followed by pain and swelling of affected site.[9]

A total of 375 samples from EPTB cases were subjected to Gene Xpert MTB/RIF Assay, which detected *M.tuberculosis* in 75 samples (20%) samples and simultaneously detected rifampicin resistance in 13(3.46%) samples. Results of our study were similar to Ya L. *et al* (2017) who subjected 414 samples for Gene Xpert and reported 18.4% (76/414) cases to be positive while 11/76 of positive cases rifampicin resistant.[10] Fuladi A. *et al* reported 49/108(45.3%) positivity by Gene Xpert on extra-pulmonary samples. [11]The higher detection rate in these studies was due to the fact that they included diagnosed cases of TB while our study was performed on TB suspects.

Maximum positivity of Gene Xpert in the present study was seen in lymph node tissues and aspirates (35.71%) followed by pus (32.88%) and gastric lavage (28.57%). Though maximum number of samples were of pleural fluid, only 13.99% (20 out of 143) samples were found to be positive. However, CSF and ascitic fluid had 12.50% and 9.83% positivity, respectively. Our results were similar to Lawn S. *et al* (2012) who, out of 268 EPTB samples reported positivity rate for tissue biopsy and fine needle aspirate 35% followed by gastric aspirates (23%), pus (21%) and CSF (5%). [7] Fuladi A. *et al* (2017) also found that MTB detection rate was higher for lymph node samples (62.7%) followed by ascitic fluid (41.6%) while as low detection rate in pleural fluid samples (31.5%).[11] Low positivity in pleural fluid samples may be because of very low number of bacilli in the sample or presence of inhibitory substance which inhibited the amplification of MTB genome without any effect on the internal control for pleural fluid samples.[12]

Of total 375 cases in present study, 306 cases were new cases. Gene Xpert detected *M.tuberculosis* in 44 (14.37%) of new cases. 51 cases had taken AKT in past and were cured of TB. Of them, 18(35.29%) were positive by Gene Xpert, suggesting relapse in these 18 cases. The relapse may be due to reactivation or exogenous re-infection. Areas where the prevalence is high, the likelihood of getting re-infection from environment is more. The major risk factor for relapse of TB are irregularity in treatment and presence of initial drug resistance. 5 (62.50%) of 8 treatment failure cases were positive. Treatment failure is common among those with previous failure due to drug resistance.[13] Positive smear at 2 months of TB treatment and poor adherence to anti TB treatment are predictors of treatment failure.[14] In our study out of 10 defaulters, 8 (80%) were positive by Gene Xpert. Few patients stopped taking AKT after 2 months because they felt better and few others because of side effects of AKT. Ravikumar *et al* (2017) reported among 224 cases of EPTB who were on DOTS treatment, 7.5% patients defaulted, saying most common reason for defaulting treatment was irregular treatment and alcohol abuse.[10]

In present study, most common co-morbid condition associated with

EPTB suspected was diabetes followed by HIV infection. In this study, 29.78% (14/47) of diabetics were positive. Our results were higher than Velingker et al (2018) who reported that 9.55% of EPTB cases had diabetes and said that diabetes increases risk of TB 3 times.[16] Xpert detected *M. tuberculosis* in 56.2% (13/23) HIV seropositive patients. Velingker A. et al found 18.7% of EPTB patients were HIV positive, which was lower than our study.[16]

In the present study, 39 (26.89%) of 145 urban slums were positive for MTB and 7 (4.82%) were rifampicin resistant by Gene Xpert, which was higher than urban non slum cases and rural areas. Alene K.A. et al reported increased TB incidence in urban settings because of poor quality housing with overcrowding, lack of water and sanitation and environmental factors.[17]

Our study showed Gene Xpert had higher sensitivity among all the tests performed Our study was in concordance with the study by Iram S. et al, who processed 40 EPTB samples and reported maximum positivity by Gene Xpert i.e 22.5% (9/40 specimens). Solid culture was positive in 10% (4/50 specimens) and ZN staining detected only 7.5% (3/40 specimens).[20]

Culture is an accepted gold standard for MTB. Nevertheless, in paucibacillary disease, the culture yield may be poor, while the molecular test have ability to detect DNA from non-viable organism with a LOD ranging between 5-100 bacilli/ml, hence may detect culture negative samples.[18,19] Low sensitivity of culture may also be attributed to killing of viable bacilli during NALC- NaOH decontamination procedure, as compared to sample processing for Xpert Assay in which better homogenization and liquefaction of sample is achieved. [10,20] Patients on anti-tubercular drugs also give negative result on culture. This could be the reason of low positivity of culture in our study.

CONCLUSION-

High degree of suspicion is required to diagnose EPTB. Conventional methods provide low sensitivity for detection of MTB in extra-pulmonary samples, as EPTB is a pauci-bacillary disease. Gene Xpert was a better test for detecting MTB in lymph node tissue and aspirates and pus and proved to be very promising technique due to its high sensitivity and specificity in diagnosis of EPTB, in smear negative and culture negative samples. Due to its automation, rapidity and simplicity, it has become an attractive tool for rapid detection of EPTB. It does not require skilled staff, sophisticated laboratories or higher biosafety facilities. Thus, it can be used in resource limited settings as point of care test. In addition to detection of MTB, Gene Xpert also detects rifampicin resistance which amplifies usefulness of this test. Rapid detection of rifampicin resistance in 2 hours will bring end to transmission of MDR-TB in community. Overall, Gene Xpert MTB/RIF Assay has good clinical application in rapid diagnosis of EPTB and rifampicin resistance which is a key to TB control strategy.

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