Original Article

# ROLE OF GENE EXPERT/MTB TEST IN CHILDHOOD TUBERCULOSIS

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## ABSTRACT:

**OBJECTIVE:** To evaluate the role of Gene Expert/MTB test in diagnosing childhood tuberculosis and resistance to Rifampicin.

**METHODS AND PATIENTS:** It was a cross sectional observational study conducted at the Department of Pediatrics, Madina Teaching Hospital, Faisalabad, during a period of 9 months i.e., July 2016 to March 2017. A total number of 65 patients suspected of having pulmonary or extra pulmonary tuberculosis on the basis of history and examination, were included in the study. They were evaluated by routine lab investigations for diagnosis of tuberculosis (CBC, ESR,Mantoux test, Xray chest, FNAC lymph nodes etc) as well as by gene expert MTB/RIF test performed on sample taken from the area of involvement. The findings were recorded on a predesigned proforma. Data was analyzed by SPSS version 23. P value <0.05 was considered significant.

**RESULTS:** Out of total 65 suspected cases, 40 turned out to be tuberculosis fulfilling the criteria of PPA scoring chart. Mean age of our patients was 9.35± 4.34. Eighteen patients (45%) showed detection of mycobacterium tuberculosis by Gene Expert/MTB/RIF assay, while 22 patients had negative result. Overall sensitivity of the test was 55 % where as specificity was 81%. Detection of mycobacterium was significantly associated with the sample site (p value = 0.003). Rifampicin resistance was not found in any of the patients.

**CONCLUSION:** Gene Xpert/MTB/RIF is quite helpful in diagnosing the childhood tuberculosis with 81% specificity but variable sensitivity depending upon the sample site. Maximum sensitivity was found for lymph node aspirate.

**KEY WORDS:** tuberculosis, gene expert/MTB/RIF, children

## INTRODUCTION:

Tuberculosis is a worldwide health problem affecting a large number of populations all over the world. This disease is more prevalent in developing countries and underprivileged population. According to the recent global estimation of tuberculosis by World Health Organization the worldwide estimated 10.4 million TB cases in 2015 and six countries accounted for 60% of this global load of tuberculosis, in descending order India, Indonesia, China, Nigeria, Pakistan and South Africa[1]. This report also ranks Pakistan among top 20 countries with highest disease burden of multidrug resistant tuberculosis[1].

Diagnosis and treatment of tuberculosis is being neglected in these countries, more so in pediatric age group. As far as the disease in children is concerned, many cases are not taken for assessment to the medical facilities by the

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family due to financial and social issues. Furthermore; even if they are taken their evaluation and confirmation of diagnosis is quite challenging due to difficulties in sample collection[2]. The culture which is considered as gold standard in diagnosing adult tuberculosis, remains negative in 20 to 80% of patients in childhood tuberculosis, where as sputum smear examination is positive in less than 10 to 15% of the cases in childhood tuberculosis[3,4]. Tuberculin skin testing is of somewhat help but it can't differentiate between active and latent tuberculosis[5]. Moreover tuberculin skin testing also carries the risk of false positive or false negative reports. Therefore diagnosis of tuberculosis in children remains a big challenge especially in extra pulmonary cases. Drug resistance may further worsen the situation.

A newly launched advance technique named Cephaid Gene Xpert MTB/RIF (Xpert) assay, can detect M. tuberculosis complex and associated Rifampin (RIF) resistance directly from clinical samples using ultrasensitive hemi-nested PCR and molecular technology[6]. This allows the detection of M. tuberculosis and RIF resistance simultaneously within 2 h and requires minimum handling and training[6]. This tool is also potentially useful for diagnosis of extrapulmonary tuberculosis as well. In December 2010 World Health Organization (WHO) endorsed the implementation of Gene expert /MTB/Rif technique for initial diagnosis of all cases in developing countries[7]. The Gene Xpert technique has been evaluated in clinical trials in adults, and children with suspected tuberculosis, and showed good sensitivity and specificity in smear-positive and culture-positive sputum samples, and proved to be reasonably accurate in smear-negative but culture-positive sputum samples[8,9,10].

Keeping in view the difficulty diagnosing childhood tuberculosis and utility of this latest technique, we performed this study to evaluate the role of gene expert in diagnosing tuberculosis in children as well as resistance to Rifampicin in our setup having either pulmonary or extra pulmonary tuberculosis.

## PATIENTS AND METHODS:

It was a cross sectional observational study conducted at the Department of Pediatrics, Madina Teaching Hospital, University Medical & Dental College, Faisalabad, during a period of 9 months i.e., 1st July 2016 to 31st March 2017.

The research proposal was approved by the Ethical Review Committee, Board of Advance Studies and Research, The University of Faisalabad. The patients presenting in the Pediatrics Department as suspected cases of Tuberculosis either pulmonary or extra pulmonary on the basis of history and examination, were included in the study. A total 65 patients were being evaluated for having tuberculosis. Informed written consent was taken from parents/caregivers. All the cases were being evaluated with laboratory investigations for tuberculosis including Complete Blood counts, ESR ,CRP, Montoux test, CXR, Fine needle aspiration cytology, sputum for AFB staining, Abdominal Ultrasonography, Pleural tap, Peritoneal Tap, CSF examination according to the indication in different patients. The sample was also sent for gene expert MTB/RIF test depending upon the organ of involvement e.g., sputum sample in case of pulmonary tuberculosis with productive cough, Lymph node aspirate, Cerebrospinal fluid, peritoneal fluid and pleural fluid in patients with extra pulmonary tuberculosis. These investigations cost was adjusted according to affordability of the patients as for non affording patients hospital zakat fund was utilized. Nutritional status of the patients was also evaluated. Diagnosis of tuberculosis was made on the basis of modified Kenneth Jones scoring criteria/Pakistan Pediatric Association Scoring chart for diagnosis of TB in children[11].

The findings were recorded on a predesigned proforma. Mycobacterium detection by Gene expert was also noted. Data was analyzed by SPSS version 23. P value <0.05 was considered significant.

## RESULTS:

A total number of 65 suspected patients were evaluated for tuberculosis out of which 40 turned out to be tuberculous as proven by Pakistan Pediatric Association scoring chart.

Minimum age of the patient was 2 years and maximum was 15 years with mean age of 9.35± 4.34.

Out of 40 tuberculous patients, 18 patients (45%) showed detection of mycobacterium tuberculosis by Gene Expert/MTB/RIF assay, while 22 patients had negative result. Sensitivity of the test was 55 % where as specificity was 81%.

The demographic profile of the patients is presented in Table 1. Majority of the BCG vaccinated patients were also suffering from tuberculosis. Mycobacterium tuberculosis detection test was performed by gene expert technique in various samples out of which the maximum positivity was found in lymph node aspirate that can be appreciated from table II, whereas the results were negative in pleural fluid and ascitic fluid aspirate. The detection of mycobacterium tuberculosis by gene expert technique has statistically significant association with the sample site (p value = 0.003). Rifampicin resistance was not found in any of the patients who had positive mycobacterium tuberculosis by gene expert technique.

**TABLE I: DEMOGRAPHIC PROFILE OF THE TUBERCULOUS PATIENTS**

| DEMOGRAPHIC FEATURES | NUMBER OF PATIENTS frequency (percentage) |
|---|---|
| Sex of the patients | |
| Male child | 26( 65) |
| Female child | 14 (35) |
| BCG vaccination | |
| Yes | 30 (75) |
| No | 10 (25) |
| Mantoux Test | |
| Positive | 14 (35) |
| Negative | 26 (65) |
| Sputum AFB | |
| Positive | 2 (5) |
| Negative | 38 (95) |

**TABLE II: MTB DETECTION BY GENE EXPERT IN DIFFERENT SAMPLESsample taken for test * MTB detected  Cross tabulation**

| | | MTB detected | | |
|---|---|---|---|---|
| | | yes | No | Total |
| sample taken for test | Sputum | 2 11.1% | 8 36.4% | 10 25.0% |
| | Lymph node aspirate | 12 66.7% | 2 9.1% | 14 35.0% |
| | CSF | 4 22.2% | 8 36.4% | 12 30.0% |
| | Pleural fluid | 0 0.0% | 2 9.1% | 2 5.0% |
| | Ascitic fluid | 0 0.0% | 2 9.1% | 2 5.0% |
| Total | | 18 100.0% | 22 100.0% | 40 100.0% |

**DISCUSSION:**

Diagnosis of tuberculosis in children is mostly done on clinical grounds all over the world in low resource countries not only due to limited resources but also due to certain diagnostic short comings[12]. Situation gets more complicated while making diagnosis of extra pulmonary tuberculosis. We found the gene expert technique 81 % specific for overall diagnosis of tuberculosis in children while sensitivity of the test was 55 %. These results can be compared with sensitivity observed by Zeka *et al*. which was 52% of overall extra pulmonary tuberculous samples[13]. The sensitivity of gene expert technique in our study was 33 % for Cerebrospinal fluid and increased uptil 86% in case of lymph node aspirate. We can compare the results with clinical validation of gene expert performed by Tortoli E et al that proved the overall sensitivity of the test in children 86%, specifity 99.8%. They also documented both sensitivity and specificity increased to 100 % for lymph node biopsy but for CSF it was 75% and 97% respectively[14]. Another study by Hillemann et al. demonstrated the effectiveness of Xpert MTB/RIF on extrapulmonary tissue and reported the

combined sensitivity and specificity as 77.3% and 98.2%[15].

In another study of children with complicated intrathoracic TB, Walter E declared the Xpert test as useful an add-on test to routine culture on bronchoalveolar lavage samples, because it enabled rapid diagnosis of tuberculosis with sensitivity of 79%[16]. As the Xpert MTB/RIF assay has greatly reduced the time of detection of mycobacterium up to two hours, it is increasingly being used for rapid diagnosis of both pulmonary TB and EPTB[17,18]. Thus it also reduces the time to initiate the antituberculous therapy from several weeks to just a few hours[19].

In our study the yield of mycobacterium was the highest in lymph node material and not a single detection could be done in pleural and ascitic fluid. Comparable results were observed by Tortoli documenting excellent sensitivity >85% in biopsy material, FNAC and CSF, while poor yield in body fluids and only seven cases were resistant to Rifampicin in their study[14].

Out of the tuberculous patients in our study, none of them was resistant to Rifampicin. Although the data regarding Rifampicin resistance in childhood tuberculosis is lacking for comparison, a latest study performed at Agha Khan University Hospital Karachi tested stool samples for MTB detection in 60 immunocompetent children suspected of tuberculosis, and no Rifampicin resistance was detected even there[20]. Another study performed in Rahim Yar khan Pakistan documented 14% resistance in pulmonary tuberculosis in adults[21]. similarly another study performed at Mayo hospital documented Rifampicin resistance in 6% of cases of adults tuberculous pleural effusion[22]. Internationally the situation may be variable, the resistance pattern depends upon the geographic distribution, like a study from Turkey documented the frequency of INH -resistant TB as 6.7 % in children and another systemic review of 95 studies documented INH resistance in 8% of cases[23,24]. The limitation of our study was relatively small sample size and limitation of sample collection. There is a need for more studies to be performed in children for documentation of patterns of resistance to antituberculous drugs.

## CONCLUSION:

Gene Xpert/MTB/RIF is a very useful technique in diagnosing the childhood tuberculosis with 100% specificity but variable sensitivity depending upon the sample site. Maximum sensitivity has been documented for tuberculous lymphadenitis. Gene expert test shortens the duration of confirmatory diagnosis and hence preventing the unnecessary delay in the treatment

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