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Original Research Article

Role of Cartridge Based Nucleic Acid Amplification Test in Paediatric

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Abstract

Introduction: Tuberculosis (TB) is a major public health problem in India and hence early diagnosis and treatment is important to prevent further complications and spread. Diagnosing in children is challenging because the easily available diagnostic tools like radiological screen which is subjective, mantoux test is a marker of exposure, not disease and due to the paucibacillary nature of mycobacterium in children. Hence there is a need of a relatively rapid and confirmative diagnostic tool. Implementation of cartridge-based nucleic acid amplification tests(CBNAAT) has augmented the detection rates and this was studied in our research in comparison with fluorescent microscopy, mantoux test and chest xray findings.

Material and Method: The present study was conducted in Government Medical College, Solapur, 200 study subjects were analyzed, pulmonary and extrapulmonary cases were tested on CBNAAT, fluorescent microscopy, mantoux test and chest xray were used in diagnosing cases.

Results: Study showed most cases were from age group 5-10years, gender distribution showed male predominance. 17.5 % cases had history of TB contact. 61.00% cases had BCG scar and most common specimen found in the study was gastric aspirate in 73.00% cases. Cases diagnosed as TB in which TB positive were 20.00% and TB negative were 80.00%. CBNAAT reported 62.50%sensitivity and 62.50% specificity.

Conclusion: CBNAAT was advantageous as it could detect cases which are missed by other conventional methods, It is a quick and requires minimal technical training to run the test. Gene Xpert MTB/RIF is a reliable technique for diagnosing extra pulmonary tuberculosis with high sensitivity and specificity.

Keywords: Gene Xpert MTB/RIF , Extrapulmonary Tuberculosis , Paucibacillary Tuberculosis , Chest X Ray , NAAT.

Introduction

In India, there are about ~400 million children who constitute about 34% of the total population. The extent of childhood TB in India is unknown due to diagnostic difficulties; it is estimated to be 10.2% of the total adult incidence. The maximum risk of a child getting TB is between 1-4 years when there is an increased risk of progression from infection to disease. Globally, about 1 million cases of pediatric TB are estimated to occur every year accounting for 10-15% of all TB; with more than 100,000 estimated deaths every year, it is one of the top 10 causes of childhood mortality. The proportion of pediatric TB cases registered under RNTCP has shown an increasing trend, from 5.6% in 2005 to 7% in 2011 [1].

Despite progress made during the last decades, the pipeline of anti-TB drug development is lagging. The need for new combination therapies to treat drug resistant forms and the need for shorter treatment regimens for all forms of TB remains urgent [2].

A major research drive has resulted in the approval by the US food and drug administration (FDA) of two new drugs for MDR TB treatment (bedaquiline and delamanid). Importantly, the mechanism of action of these drugs differs from that of existing TB drugs. The use of nucleic acid amplification tests (NAAT) for TB diagnosis has greatly increased following the endorsement by WHO in 2010 of the Gene Xpert MTB/RIF test. This test allows the rapid detection of both MTB and resistance to rifampicin, but has a number of important limitations, which stop it being a true point-of-care test [3-6].

A series of meta-analyses have shown cartridge based nucleic acid amplification test (CBNAAT)/ Xpert MTB/ RIF to have a high specificity with variable sensitivity in different type of specimens for TB diagnosis. In 2013, the WHO endorsed the use of CBNAAT for TB diagnosis in pediatric presumptive pulmonary and extra-pulmonary tuberculosis (EPTB) cases. CBNAAT, a tool with a quick turn-around time, which simultaneously detects TB and rifampicin resistance, offers a promising solution to achieve the global objective of improved TB care and control and early TB case detection [7]. We aimed to study the diagnostic usefulness of CBNAAT (sensitivity, specificity, positive predictive value and negative predictive value) for diagnosing pediatric tuberculosis in our hospital setting. [8-10]

Material and Methods

It is a Prospective Observational Study done after approval from Ethical Committee of Tertiary Care Centre & Government Medical College. Data was collected from all patients attending Tertiary Care Centre inPaediatrics OPD who got admitted in Paediatrics ward or PICU, irrespective of their gender/ background /socio economic status and undergone various operative interventions.

Method of sampling was non-random, purposive, child was considered to be a case of diagnosed tuberculosis based on microbiological evidence i.e. CBNAAT and / or by Fluorescentmicroscopy or clinical evidence with supporting evidence of skin test or radiological findings or imaging findings or pathological findings .

Following are the clinical or investigational suspicion of Tuberculosis :

Fever and or cough > 2 weeks, history of weight loss or no weight gain in past 3 months (loss of weight defined as loss of more than 5% body weight as compared to highest weight in last 3 months), hilar lymphadenopathy on chest skiagram, Tuberculin skin test positivity, coexistence of precipitating illness, abdominal USG suggestive of tuberculosis, ADA positivity in pleural fluid,CSF findings consistent with Tubercular Meningitis, Cranial CT suggestive of Tuberculoma.

Data collection sheets were filled in by the investigator. All of the factors related to the patient were noted. Consent as obtained from parents or legal guardian. After completing the collection of data it was compiled in a systematic way. Data analysis was done both manually and by using computer. Calculated data were arranged in systemic manner, presented in various table and figures and statistical analysis was made to evaluate the objectives of this study with the help of Statistical Package for Social Science (SPSS) version 21.

p-value < 0.05 is considered statistically significant.

Results

Study showed most cases were from age group 5-10 years were 82 (41.00%) followed byage 1 month–5 years were 62 (31.00%) followed by 10- 14 years were 56 (28.00%).

Table 1: Distribution of cases based on various factors.

Variables	No. of patients	Percentage
Age distribution		
1 mon-5yrs	62	31.00
5ys-10 yrs	82	41.00
10yrs-14yrs	56	28.00
Sex distribution		
Male	125	62.50
Female	75	37.50
History of contact		
Yes	35	17.5
No	165	82.5
BCG Scar		
Present	122	61.00
Absent	88	39.00
Types of specimens		
Gastric aspirate	146	73.00
Sputum	15	07.50
Ascitic fluid	04	02.00
Pleural fluid	10	05.00
CSF	16	08.00
Tracheal aspirate	00	00.00
Lymph node aspirate	09	04.50
Bronchoalveolar lavage	00	00.00
Distribution of pediatric patients according to clinical features.		
Cough(>2 weeks)	195	97.50
Fever(low grade(99.5^0F),evening rise)	162	81.00
Weight loss / poor weight gain	121	60.50
Breathlessness	22	11.00
Headache with fever	23	11.50
Lymphadenopathy	09	04.50
Abdominal pain	03	01.50
Convulsion	02	01.00
Altered Mental Status	03	01.50
Nausea and Vomiting	02	01.00

Bowel Disturbances	02	01.00
Head heaviness/Meningeal signs	03	01.50
Based on CBNAAT		
Positive	25	00
Negative	15	160
Pulmonary & extra-pulmonary TB based on CBNAAT		
Pulmonary TB	10	40
Extra-pulmonary TB	15	60
According to extra-pulmonary TB		
TB meningitis	04	26.66
TB lymphadenitis	06	40.00
TB causing Pleuraleffusion	05	33.33
Abdominal TB	00	00

Gender distribution showed male predominance i.e. 125(62.50%) and female cases were 75(37.5%). Distribution of study subjects according to history of TB contact, was present in 35(17.5 %) cases and there was no history of contact in 135(82.5%) cases.

Distribution of study cases according to BCG scar, which was seen in 122(61.00%) cases and there was no scar seen in 88(39.00%)cases. Most common specimen found in the study was gastric aspirate in 146(73.00%) cases, followed by CSF in 16(08.00%), followed by Sputum in 15(07.50%) cases.

Most common sign and symptom was cough (>2 weeks) in 195(97.50%) cases, followed by fever in 162 (81.00%) cases, weight loss or poor weight gain was seen in 121(60.50%) cases.Cases diagnosed as TB in which TB positive were 40(20.00%) and TB negative were 160 (80.00%).

Table 2: Results of diagnostic test by final diagnosis.

Test	TB	Non-TB
Study cases based on CBNAAT		
Positive	25	00
Negative	15	160
Study cases based on Mantoux		
Positive	32	07
Negative	08	153
Study cases based on Chest xray		
Positive	27	10
Negative	13	150
Study cases based on Fluorescent microscopy		
Positive	07	02
Negative	33	158

In our study, TB positive caseswere 20.00%, similar tour study PotdarP Setal[10] (2016), reported extra-pulmonary TB 60.87% in comparison to Pulmonary TBi.e.39.13%.

Table 3: Comparison of various diagnostic accuracy in detecting TB.

Test (N=200)	Sensitivity %	Specificity%	PPV %	NPV %
CBNAAT	62.50	100	100	98.62
Mantoux test	80	95.6	40.6	99.2
Chest X-ray	67.5	93.8	28.7	98.7
Fluorescentmicroscopy	17.5	98.8	34.3	97

PPV- PositivePredictiveValue; NPV- NegativePredictiveValue

Results of CBNAAT testing by final diagnosis and found that sensitivity was 62.50 % and specificity was 100.00% and Positive Predictive Value and Negative Predictive Value were 100.00% and 98.62% respectively. Similarly Bates M et al [16] (2013) and Detjen AK et al [17] (2015) showed CBNAA Thaving Sensitivity: 68.8%, Specificity: 99.3% and Sensitivity: 66%, Specificity: 98% respectively. Pulmonary tuberculosis cases based on CBN AAT were 10 (40%) and extra-pulmonary tuberculosis cases were 15 (60%), similarly PotdarP Setal [10] (2016), shows same comparison as 63.03 to 46.97. Most common extra pulmonary tuberculosis in present study is tubercular lymphadenitis in 06 (40.00%) cases. Similarly Potdar PS et al [10] (2016), showed tuberculous lymphadenitis (26.5%) was the commonest form for allages followed by abdominal Koch'sin 9.1% and Maltezou HC et al [18] (2000), showed that lymphadenitis (47%) was the most common manifestation. Results of Mantoux testing by final diagnosis and found that sensitivity was 80.00% and specificity was 95.62% and Positive Predictive Value was 40.58% and Negative Predictive Value was 99.22%. Similarly Anuradha G et al [12] (2019) showed that the Mantoux screeningtest was reactive in 85 (68.5%) patients and Sreerama reddy CT etal [19] (2010), in their study on tuberculosis ad a Mantoux positivity of 66%in pulmonary group with higher Mantoux positivity of 71% in extra pulmonary form of tuberculosis. Results of chest X-ray testing by final diagnosis and found that sensitivity 67.50%and specificity was 93 .75% and Positive Predictive Value was 28. 74% and Negative Predictive Value was 98.72%. Similarly Anuradha G et al [12] (2019) showed positive chest Xray findings were noticed in 93.5% of pulmonary tuberculosis patients and all children with disseminated TB. Non Homogenous Opacities, consolidation, pleural effusion, hilar nodes, bronchiectasis and military pattern are the chest X-ray findings notice din their patients. Results off luorescent micros copy testing by final diagnosis and found that sensitivity was 17.50% and specificity was 98.75%and Positive Predictive Value was 34.33% and Negative Predictive Value was 96.97%. Similar to present study Arora Aetal [11] (2018) showed fluorescent micros copy Sensitivity: 47.6%, Specificity: 98.7% but Very contrast to present study Sree kanth Betal [20] (2020), showed that fluorescent microscopy testing was done for 337 samples of the patients who were having a history suggestive of PTB. Out of these, 36 (10.68%) sputum samples were AFB positive and 301(89.3%) were negative

Table 4: Results with respect to different statistics- CBNAAT compared to other diagnostic test.

CBNAAT With >>	Mantoux	Chest xray	Fluorescent microscopy
Pooled Sensitivity	41.03	56.76	22.22

Pooled Specificity	94.41	97.55	87.96
PPV	21.51	46.34	22.22
NPV	97.72	98.37	87.96

PPV- PositivePredictiveValue; NPV- NegativePredictiveValue

Comparison between CBNAAT and Fluorescence Microscopy findings and found that pooled sensitivity was 22.22% and specificity was 87.96% and Positive Predictive Value and Negative Predictive Value were 6.45% and 96.80 % respectively. [8] But Kumar A et al [7] (2018) showed sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT in reference to ZN smear are 100%, 90.68%, 71.42% and 100% respectively. Sensitivity of conventional sputum smear microscopy by ZN staining was very low (10.68%). Similar to present study Geleta DA etal [21] (2015) have found a very low sensitivity (9.3%) of sputum smear for AFB. Sensitivity of CBNAAT varied significantly between 100% in sputum smear-positive PTB and 15.38% in sputum smear-negative PTB. In studies conducted by Mukherjee S et al [22] (2017) and Geleta DAet al [21] (2015) showed similar results of very high sensitivity of CBNAAT in smear positive cases have been reported. Mohanty T et a [23] (2014) and Dewan Retal [24] (2015) reported sensitivity of 32% and 32.58% of CBNAAT in smear negative PTB, which correlates with present study. Comparison between CBNAAT and chest Xray findings and found that pooled sensitivity was 56.76% and specificity was97.55% and Positive Predictive Value was 46.34 % and Negative Predictive Value was 98.37%. In contrast to present study Anuradha G et al12(2019) showed positive chest X ray findings were noticed in 93.5% of pulmonary tuberculosis patients and all children with disseminated TB. Mukherjee Setal [22] (2017) and Geleta DA et al [21] (2015) showed similar results of very high sensitivity of CBNAAT in smear positive cases have been reported in comparison to chest Xray findings. Comparison between CBNAAT and Mantouxtest findings and found that pooled sensitivity was 41.03% and specificity was 94.41% and Positive Predictive Value was 21.51% and Negative Predictive Valuewas97.72%. Similar topresent study Anuradha Getal [12] (2019)showed Mantoux reactivity was noted in 66.7% children with pulmonary TB, 75% of extra pulmonary TB and 60% of patients in disseminated group in comparison to CBNAAT i.e. >95% in studies like Mukherjee S et al [22] (2017) and Geleta DAetal [21] (2015). [25]

Discussion

Observational Study done in Department of Paediatrics, Government Medical College & Tertiary Care Centre from October 2018 to September 2020 in asamplesizeof200 cases that fulfill inclusion criteria. The age distribution showed that most cases were from age group 5-10 years were 41.00%, very similar to our study Padmaja GV et al [8] (2019) showed majority of the cases were from the age group 5-10 years i.79 (45.67%) but in contrast to the present study Verma J et al [9] (2014) showed approximately 70 % of study group was distributed in 2- 5 years of age group. In our present study we found Mean±SD of age was 07.031±03. PotdarPSet al [10] (2016), mean age of the study subjects was10.58 years. Our study reported male predominance, similarly Arora A et al [11] (2018) found 55% males and 45% females, Verma J et al9 (2014) also found Male to female ratio was 1.7:1. Distribution of study cases according to h/o TB contact was 17.5 %, in contrast to present study Anuradha Goyal [12] (2019) study showed that 51.61% of cases having h/o TB contact. BCG scar was seen in 61.00%, similarly Anuradha G et al [12] (2019) study showed that most caseshaving BCG scari.e.84.67%.

Most common specimen found in the study was gastric aspirate 73.00%, followed by CSF in 8.00%,Similar to our study Abinaya S et al [13] (2018) showed among the specimens collected, most ofthem were pulmonary samples n=106 (92.1%). Among them, gastric aspirates (57.4%) were predominant. The extra pulmonary samples n=9 constitute 7.9% of the total samples collected. In our study most common sign and symptom was cough (>2 weeks) in 97.50%, followed by fever in 81.00% Similarly Seth V et al [14] (1993)were fever was reported in 65.3 % cases, but in contrast studies like Verma J et al [9] (2014), shows that fever (96.1%) was the most common presenting symptom followed by anorexia in 66.2% cases and Bai SS et al15(2014), study found fever in only 28.6%.

Conclusion

This work was done to find out ideal diagnostic methods for diagnosis of tuberculosis among children. Among the methods which were used for the diagnosis of paediatric tuberculosis, CBNAAT was advantageous as it could detect more cases which are missed by other conventional methods.

The Xpert MTB/RIF assay is a new test that is revolutionizing tuberculosis (TB) control by contributing to the rapid diagnosis of TB disease and drug resistance. Major advantages of the Xpert MTB/RIF assay are that results are available quickly, and minimal technical training is required to runthe test. Additionally, the Xpert MTB/RIF assay can quickly identify possible multidrug-resistant TB. From our study we conclude that Gene Xpert MTB/RIF is simple and reliable technique for diagnosing extra pulmonary tuberculosis with high sensitivity and specificity not only in smear positive cases but also insmear negative cases. It is a game changer not only in pulmonary tuberculosis control but probably also in extra pulmonary tuberculosis.

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