Original Research Article

Utility of GeneXpert MTB/RIF Assay in diagnosing Paediatric Tuberculosis

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Abstract

Background: Childhood tuberculosis (TB) reflects recent transmission of infection and hence its burden provides an accurate measure of level of Tuberculosis control achieved in a particular community.Non availability of practical gold standard method makes the diagnosis of childhood tuberculosis complicated. Bacteriological confirmation is rarely achieved due to predominant paucibacillary nature of childhood tuberculosis. Sputum microscopy is the only test available in endemic areas but is positive in less than 10 – 15% of children with probable tuberculosis. culture yields are usually low. World Health Organization (WHO) recommends the use of GeneXpert system that gives results in less than two hours. In this study, we have used GeneXpert MTB/RIF Assay for diagnosis of Paediatric Tuberculosis and Rifampicin resistance. **Objectives:** of the study were 1) To study the occurrence of tuberculosis in Paediatric age group. 2) To study the age and sex predominance of Tuberculosis. 3) To study occurrence of MDR-TB (Multi Drug Resistant) in paediatric patients. **Materials and Methods:** Sample testing was done using GeneXpert MTB/RIF Assay system from June 2018-December 2019. Total 179 samples were suspected for Paediatric Tuberculosis. **Results:** 11.15% samples were positive for Tuberculosis by GeneXpert Assay. 9-12 years age group was most commonly affected (60%). Males were more affected than females (2:1). Sputum was the most common sample obtained. MDR Tuberculosis was seen in 10% cases.

Key Words: Paediatric, Gastric lavage, GeneXpert, MDR-TB.

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Received Date: 13/12/2019 Revised Date: 10/01/2020 Accepted Date: 03/02/2020
DOI: https://doi.org/10.26611/10081332

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 03 March 2019

INTRODUCTION

Globally, it is estimated that over 5,50,000 children suffer from Tuberculosis every year and many die unnecessarily from this fully curable disease. In India, there are about ~400 million children who constitute about 34% of total population.[1] Tuberculosis (TB) is an important cause of childhood morbidity and mortality.[2] Prevalence of childhood tuberculosis has been reported to be between 3 and 25% in different countries.[3,4] Lower importance is given to childhood tuberculosis due to difficulty in diagnosis, asymptomatic disease gets frequently ignored, few cases in number, children are considered as non-contagious in most of the cases and due to assumption that effective control of tuberculosis with BCG Vaccination by itself could effectively control childhood tuberculosis.[5] Infected children represent main reservoir of *Mycobacterium tuberculosis* (*Mtb*) and serve as potential future cases.Extra Pulmonary tuberculosis implies isolated occurrence of tuberculosis at body sites other than lungs. Because appropriate specimens might be difficult to obtain from extra-pulmonary sites, and the number of bacilli is generally low, the bacteriological confirmation of Extra pulmonary Tuberculosis is often more difficult than pulmonary tuberculosis. Microscopy is of little value in children, who typically have paucibacillary tuberculosis and have difficulty in producing sputum.[6] Though culture on Lowenstein-Jensen (LJ) medium is gold standard, it takes several

How to cite this article: Shiva Shankari L, Nitin A Ambhore. Utility of GeneXpert MTB/RIF assay in diagnosing paediatric tuberculosis. *MedPulse International Journal of Microbiology.* March 2020;13(3): 19-22. https://www.medpulse.in/Microbiology/

weeks to give result, which delays the treatment of patient[7]. Molecular methods like polymerase chain reaction (PCR) are available only at few institutions. World Health Organization(WHO) in December 2010 recommended use of a new Cartridge-Based Nucleic Acid Amplification Test(CB-NAAT) named GeneXpert system[8]. The Gene Xpert MTB/RIF assay employs five distinct molecular beacons (nucleic acid probes), each labelled with a differentially coloured fluorophore and responding to a specific nucleic acid sequence within the *rpoB*gene of *M.tuberculosis*.[9] This system detects tuberculosis along with Rifampicin resistance. The time taken is less than 2 hours and directly untreated sputum samples can be used. Revised National Tuberculosis Control Programme (RNTCP) is also currently using GeneXpert MTB/RIF Assay to diagnose Pulmonary and Extra pulmonary TB. [8] In the present study, We have used GeneXpert MTB/RIF Assay for diagnosis of Paediatric tuberculosis along with simultaneous detection of Rifampicin resistance.

MATERIAL AND METHODS

A retrospective study was conducted from 1 June 2018 to 31 December 2019 in Department of Microbiology, GMC Akola, Maharashtra. RNTCP programme is being carried out at our Institution. Samples were collected under this programme and analysed by GeneXpert. Early morning deeply expectorated sputum samples were collected from all clinically suspected cases in sterile wide mouth containers after taking consent from the patient. Extra pulmonary samples were collected depending on the sites involved. The samples were subjected to GeneXpert MTB/ RIF Assay manufactured by Cepheid, France for the detection of *M. tuberculosis* and then rifampicin resistance in them. GeneXpert MTB/RIF is a cartridge-based nucleic acid amplification technique which includes semi-quantitative, nested real-time PCR in vitro diagnostic test for the detection of MTBC DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputum. RIF-resistance associated mutations of the rpoB gene in the samples from patients at risk for rifampicin resistance. As per manufacturer's guidelines, 2 ml of sample reagent is added to 1 ml of each fresh sample directly into collection container. The lid was replaced and shaken vigorously 10–20 times. It was incubated at room temperature. After 10 minutes of incubation, the specimen was shaken vigorously 10–20 times. It was incubated for 5 minutes again. The sample was ensured perfectly fluid before processing with no more clumps of sputum. If still found viscous, further waiting for 5–10 minutes was done before processing it in the cartridge. At least 2 ml of processed sample was taken with plastic transfer pipette from the collection container to single use, disposable, self contained GeneXpert cartridge. Then, it was subjected to GeneXpert MTB/RIF to create a test. After scanning the cartridge barcode, loading was done on blinking module. The results were visualised on the attached computer and interpreted by using software. The GeneXpert MTB/RIF was repeated on the second sample if it has shown indeterminate susceptibility to RIF.

RESULTS

Total 179 samples were collected from 1st June 2018 to 31st December 2019 in Paediatric age group (upto 12 years) at GMC, Akola.

Table 1: Distribution of Samples from different anatomical sites

Type of Sample	Distribution
Sputum	115(64.24%)
Gastric Lavage	26(14.52%)
CSF	14(7.82%)
Pleural Fluid	24(13.40%)
Total	**179(100%)**

Table 2: Age and sex distribution in clinically suspected tuberculosis cases

	0-4 years	5-8 years	9-12 years
Males	40(22.34%)	34(18.99%)	39(21.80%)
Females	11(6.15%)	21(11.73%)	34(18.99%)
Total (179)	**51(28.49%)**	**55(30.72%)**	**73(40.79%)**

Table 3: Age and sex distribution in tuberculosis positive cases by GeneXpert Assay

	0-4 years	5-8 years	9-12 years	Total
Males	2(10%)	3(15%)	9(45%)	**14(70%)**
Females	-	3(15%)	3(15%)	**6(30%)**
Total(20)	**2**(10%)	**6(30%)**	**12(60%)**	**20(100%)**

Table 4: Type of sample positive and negative by GeneXpert Assay

Sample	Positive by GeneXpert	Negative by GeneXpert
Sputum	18(10.05%)	97(54.18%)
Gastric lavage	1(0.55%)	25(13.96%)
CSF	-	14(7.87%)
Pleural fluid	1(0.55%)	23(12.84%)
Total (179)	**20(11.15%)**	**159(88.85%)**

Table 5: Rifampicin sensitive and resistance cases among Tuberculosis positive cases

Rifampicin resistance	Rifampicin sensitive	Total
2(10%)	**18(90%)**	**20(100%)**

DISCUSSION

In present study conducted from June 2018 – December 2019, there were 179 clinically suspected paediatric patients (<12 years) having Tuberculosis (Table 1). Out of 179 patients, 28.49% were in 0 - 4 years age group, 30.72% were in 5-8 years age group and 40.79% were in

9 – 12 years age group. In our study, 20 samples (11.15%) were detected positive for *M.tuberculosis* by GeneXpert system. This correlates with the study of Anne *et al.* in which occurrence of Tuberculosis was 11% by GeneXpert system.[6] In the study of Reena *et al.*, which was conducted for 13 years, there were 14849 clinically suspected cases and 15.9% were positive which correlates approximately with our study.[10] Incidence of tuberculosis was found to be 3.4% in the study of Abhijeet Mukherjee *et al.*.[11] In the study of Verma *et al.*, conducted for 6 months, there were 330 clinically suspected cases for tuberculosis and 30.90% were found positive. But in this study, adult patients were also included. [12] In present study according to table no:3, 2(10%) cases were positive in 0-4 years age group, 6(30%) were positive in 5-8 years age group and 12(60%) were positive in 9-12 years age group. Maximum number of cases were from 9 – 12 years age group. This correlates with the study of Gandra *et al.*, in which 8.96(±) 2.83 years was most commonly involved.[13] This is in contrast to study of Irvane *et al.* from Aurangabad in which maximum number of cases were in 0 – 5 years(69.56%).[14] The reason could be due to more maternal Tuberculosis. In present study, 14(70%) males were positive for tuberculosis and 6(30%) females were positive. This correlates approximately with the study of Gandra *et al.* in which 61.65% cases were males.[13] This also correlates with study of Irvane *et al.*, Mousa *et al.* Rama Prakash *et al.* in which 78.20%, 60% and 51.52% were males respectively.[14,15,16] More incidence in males could be due to high exposure to outside atmosphere. In present study as per Table 4, maximum samples were sputum followed by gastric lavage and pleural fluid. No CSF samples were positive. This correlates with the study of Verma *et al.* in which maximum samples were Sputum, followed by gastric lavage.[12] In the study of Gandra *et al.* also, maximum samples were sputum samples. [13] In the study of Giang *et al.*, there were maximum gastric lavage samples. [17] The reason could be the median age of patients were 18 months. In these patients, sputum sample is difficult to obtain so gastric lavage is commonly used. In our study, maximum patients were in 5 – 12 years age group. In these patients, we could get sputum samples quite easily. In our study, Multi Drug Resistant Tuberculosis(MDR TB) cases were 10% while 90% were Rifampicin sensitive (Table 5). This approximately correlates with study of Azger *et al.* in which 19% cases were MDR. [18]. In study of Verma *et al.*, 22.54% cases were Rifampicin resistant. [12]

CONCLUSION

GeneXpert has short turn-around time and simultaneously detects *M.tuberculosis* and Rifampicin resistance, a marker for Multi Drug Resistant (MDR) strains in less than 2 hours which is much earlier than conventional LJ culture. On-demand rapid results make infected patients to be in isolation efficiently and receive treatment with the appropriate therapeutics effectively. Highly sensitive for smear positive and negative samples due to robust mechanical DNA extraction procedure. Uses 3 specific primers and 5 unique molecular probes to ensure a high degree of specificity. Assay targets *rpoB* gene, which is critical for identifying mutations associated with Rifampicin resistance. GeneXpert assay is easy to perform and is less dependent on user's skills. Because it is closed system, biosafety and contamination concerns are minimized.

LIMITATIONS OF THE STUDY

Comparison between sputum microscopy and culture with GeneXpert system was not done. HIV positives and negatives were not included.

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Source of Support: None Declared
Conflict of Interest: None Declared