Use of geneXpert in the diagnosis of paediatric tuberculosis – A retrospective study in Northern Maharashtra

Sunil P. Lilani[1], Pooja G. Shah[2,*], Madhuri M. Suryawanshi[3], M. N. Dravid[4]

[1]Associate Professor, [2]Tutor, [3]Assistant Professor, [4]Professor & HOD, [1-4]Dept. of Microbiology, [1,3,4]Shri Bhausaheb Hire Govt. Medical College, Dhule, Maharashtra, [2]Government Medical College, Akola, Maharashtra, India

***Corresponding Author:**Dr. Pooja G. Shah
Email: poojashah1688@gmail.com

Abstract

Introduction: Tuberculosis (TB) is an important cause of childhood morbidity and mortality. Prevalence of childhood TB has been reported to be between 3 and 25% in different countries. GeneXpert gives results in approximately 4 hours and hence it is widely used.

Aims and objectives:

1. To study the occurrence of tuberculosis in pediatric patients.
2. To study the age and sex predominance of TB.
3. To study occurrence of MDR-TB(Multi Drug Resistant) in pediatric age group.

Material and Methods: Sample testing was done using GeneXpert system as per manufacturer's guidelines from January 2016-December 2017.

Results: Total 186 samples were suspected for pediatric TB from January 2016-December 2017. 11.48% samples were positive for TB by geneXpert. 9-12 years age group was most commonly affected(57.14%). Males were more affected than females(2:1). Sputum sample was most common sample obtained followed by gastric lavage and pleural fluid. MDR TB was seen in 14.29% cases.

Conclusion: GeneXpert proves to be important method in detection of tuberculosis with early results.

Keywords: GeneXpert, MDR-TB, 2016-2017, Pediatric.

Introduction

Tuberculosis (TB) is an important cause of childhood morbidity and mortality.[1]In India, there are about ~400 million children who constitute about 34% of the total population.[2]Prevalence of childhood TB has been reported to be between 3 and 25% in different countries.[3,4]Lower importance is given to childhood T.B as compared to the adult form because it is difficult to diagnose, few cases in number and due to assumption that effective control of TB with BCG by itself could effectively control childhood TB.[5]The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung. Because appropriate specimens might be difficult to obtain from extra-pulmonary sites, and the number of bacilli is generally low, the bacteriological confirmation ofextra pulmonary TB is often more difficult than for pulmonary tuberculosis. Microscopy is of little value in children, who typically have paucibacillary tuberculosis and have difficulty producing sputum.[6] Though culture on Lowenstein-Jensen (LJ) medium is gold standard, it takes several weeks to give the result, which delayed the treatment of patient.[7]Molecular methods like polymerase chain reaction (PCR) are available only at few institutes.WHO in December 2010 has recommended use of a new cartridge-based nucleic acid amplification test (CB-NAAT) named GeneXpert system.[8] The 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 can detect TB along with rifampicin resistance. The time taken is less than 2 h and directly untreated sputum samples can be used. Revised National TB Control Programme (RNTCP) is also currently using Xpert MTB/RIF to diagnose pulmonary and extra pulmonary TB.[8]We have currently used GeneXpert system for the diagnosis of paediatric tuberculosis.

Material and Methods

RNTCP programme is being carried out at Shri Bhausaheb Hire Govt. Medical College and tertiary care hospital, Dhule, Maharashtra. Samples were collected under this programme and were analysed by GeneXpert. A retrospective study was conducted from 1 January 2016 to 31 December 2017. Early morning deeply expectorated sputum samples were collected from all clinically suspected cases in sterile wide mouth containers after taking consent from the patient. Extrapulmonary samples were collected depending on the sites involved. The samples were subjected to GeneXpert MTB/ RIF 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 perfectly fluid before being processed with no more clumps of sputum. If still it is viscous, we waited for 5–10 minutes further 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 186 samples were collected from 1st January 2016 to 31st December 2017 in paediatric age group (upto 12 years) at SBH GMC, Dhule. The age and sex distribution is as follows:

Table 1: Age and sex distribution in clinically suspected tuberculosis cases

	0-4 years	5-8 years	9-12 years
Males	42(22.57%)	35(18.82%)	34(18.28%)
Females	14(7.53%)	22(11.83%)	39(20.97%)
Total (186)	56(30.10%)	57(30.65%)	73(39.25%)

Table 2: Age and sex distribution in tuberculosis positive cases by GeneXpert

	0-4 years	5-8 years	9-12 years	Total
Males	2(9.52 %)	3(14.28 %)	9(42.86%)	14(66.67 %)
Females	-	4(19.06 %)	3(14.28%)	7(33.33%)
Total(21)	2(9.52 %)	7(33.34 %)	12(57.14 %)	21(100%)

Table 3: Type of sample positive and negative by geneXpert

Sample	Positive by GeneXpert	Negative by GeneXpert
Sputum	19(10.21%)	99(53.22%)
Gastric lavage	1(0.54%)	25(13.44%)
CSF	-	23(12.37%)
Pleural fluid	1(0.54%)	18(9.68%)
Total	21(11.29%)	165(88.71%)

Table 4: Rifampicin sensitive and resistance cases amongst tuberculosis positive cases

Rifampicin resistance	Rifampicin sensitive	Total
3(14.29%)	18(85.71%)	21(100%)

Discussion

In our study which was conducted from January 2016 – December 2017, there were 186 clinically suspected paediatricpatients (<12 years) having TB (Table 1). Out of 186 patients, 30.10% were in 0-4 years age group, 30.65% were in 5-8 years age group and 39.25% were in 9 – 12years age group. In our study, 21 samples (11.48%) were positive for M.tb by GeneXpert system. This correlates with the study of Anne et al in which occurrence of TB 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 TB was found 3.4% in the study of Abhijeet Mukherjee et al.[11]In the study of Verma et al, which was conducted for 6 months, there were 330 clinically suspected cases for tuberculosis and 30.90% were positive for TB. But in this study, adult patients were also included.[12]

In our study as per table no. 2, 2(9.52%) cases were positive in 0-4 years age group, 7(33.34%) were positive in 5-8 years age group and 12(57.14%) 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 TB.

In present study, 14(66.67%) males were positive for TB and 7(33.33%) females were positive. This correlates with 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.[14-16]More incidence of males could be due to more exposure to outside atmosphere.

In our study as per table 3, 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 in the study of Giang et al, the median age of patients was 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 can get sputum samples quite easily.

In our study, MDR TB cases were 14.29% while 85.71% were Rifampicin sensitive (Table 4). This 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 proves to be important method in detection of tuberculosis. We get result in 3 – 4 hours which is much earlier as compared to conventional LJ culture.

Limitations

We didn't compare sputum microscopy and culture with GeneXpert system. HIV positives and negatives were not included.

Acknowledgement

All authors are thankful to Mrs. Manisha Teli and department of microbiology for their support throughout the study.

References

1. Kabra SK, Lodha R, Seth V. Category based treatment of tuberculosis in children. Indian Pediatr 2004;41:927-37.
2. The Registrar General & Census Commissioner, India, New Delhi, Ministry of Home Affairs, Government of India.(online)2001. Available from: http://www.censusindia.gov.in/Census_Data_2001/India_at_Glance/broad.aspx.
3. Stop TB. Partnership childhood TB, subgroup world health organization: Guidance for national tuberculosis programmes on the management of tuberculosis in children. Chapter 1: Introduction and diagnosis of tuberculosis in children. Int J Tuberc Lung Dis 2006;10:1091-97.
4. Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. Int J Tuberc Lung Dis 2004;8:636-47.
5. Kumar A, Gupta D, Nagaraja SB, Singh V, Sethi GR, et al. Updated national guidelines for pediatric tuberculosis in India. Indian Pediatr 2012;50:301-06.
6. Anne K Detjen, Andrew R DiNardo, Jacinta Leyden, Karen R Steingart, Dick Menzies. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med 2015;3(6):451–61.
7. Walusimbi S, Bwanga F, De Costa A, Haile M, Joloba M, Hoffner S. Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infectious Dis 2013;13:507.
8. Tuberculosis. WHO Global Tuberculosis Report 2014. http://www.who.int/ tb/ publications/factsheet global.pdf.
9. Weyer K, Mirzayev F, Migliori GB, Gemert WV, D'Ambrosio L, Zignol M. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. ERJ 2013;42:252–71.
10. Reena Raveendran, Jaswinder Kaur Oberoi & Chand Wattal. Multidrug-resistant pulmonary & extrapulmonary tuberculosis: A 13 years retrospective hospital-based analysis. Indian J Med Res 2015;142:575-82.
11. Mukherjee A, Chowdhury R, Singla R, Saha I, Dutta R, Das T. Comparison between childhood and adult tuberculosis in a rural tuberculosis unit of West Bengal: A retrospective study. Lung India 2014;31:116-20.
12. Verma D, Sharma R, Chand A.E, Saxena A. The application of gene xpert for the diagnosis of mycobacterium tuberculosis and MDR TB in Kota region. Int J Med Res Rev 2016;4(10):1863-70.
13. Gandra NR, Gali JH. GeneXpert: a game changer in the detection and diagnosis of childhood tuberculosis. Int J Contemp Pediatr 2018;5:35-41.
14. Iravane J.A., et al., (2017) GeneXpert for Gastric Lavage to Diagnose Pulmonary Tuberculosis in Children. Int J Microbiol Res 2017;9(11):966-69.
15. Husseiny Sh. Moussa, Faten S. Bayoumi, Ahmed M. Ali. Evaluation of GeneXpert MTB/RIF assay for direct diagnosis of pulmonary tuberculosis. Saudi Med J 2016;37(10),1076-81.
16. S Rama Prakasha, G Suresh, Ivor Peter D'sa, Shobha S Shetty,S Ganesh Kumar. Mapping the Pattern and Trends of Extrapulmonary Tuberculosis.J Glob Infect Dis 2013; 5(2):54–9.
17. Giang DC, Duong TN, Ninh HA DT, Nhan HT, Wolbers M, Nhu NTQ et al. Prospective evaluation of GeneXpert for the diagnosis of HIV-negative pediatric TB cases. BMC Infectious diseases 2015.
18. Azger Dusthackeer, Gomathi Sekar, Shambhavi Chidambaram, Vanaja Kumar, Pranav Mehta, Soumya Swaminathan. Drug resistance among extrapulmonary TB patients: Six years experience from a supranational reference laboratory.Indian J Med Res 2015;142:568-657.

Conflict of interest: None

Funding: None

Cite this Article as:
Lilani S, Shah P, Suryawanshi M, Dravid M. Use of geneXpert in the diagnosis of paediatric tuberculosis – A retrospective study in Northern Maharashtra. Natl J Integr Res Med 2018; 9(5):20-22