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Utility of GeneXpert in Diagnosis of Pediatric Tuberculosis and Detection of Rifampicin Resistance

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ABSTRACT

Background: Tuberculosis (TB) remains a significant public health challenge in developing countries, including India, where children under 15 years account for 12% of all TB cases. The National Tuberculosis Elimination Programme (NTEP) recommends the use of GeneXpert/Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) for TB diagnosis, followed by Line Probe Assay (LPA) for detecting drug resistance. This study aimed to determine the prevalence of TB and rifampicin resistance in pediatric patients and to evaluate the utility of CBNAAT in comparison with LPA and Ziehl-Neelsen staining. **Methods**: This retrospective study was conducted over one year, from 1st January 2022 to 31st December 2022, at a tertiary care hospital in western Maharashtra. A total of 528 samples from pediatric patients (aged <18 years) suspected of having TB were included. Samples underwent CBNAAT testing and Ziehl-Neelsen staining. Positive samples were further analyzed using LPA or Mycobacterial Growth Indicator Tube (MGIT) culture. **Results**: Among the 528 samples analyzed, gastric lavage accounted for 81.25% of the specimens. CBNAAT detected TB in 61 (11.55%) of the samples, with 15 (24.59%) of these showing rifampicin resistance. Samples with very low or low bacterial loads were often missed by Ziehl-Neelsen staining. **Conclusion**: Pediatric tuberculosis continues to be a public health concern in developing countries. The study found an 11.55% positivity rate in children under 18 years of age, with 24.59% of positive cases exhibiting rifampicin resistance. The findings underscore the importance of using advanced diagnostic techniques like CBNAAT and highlight the limitations of Ziehl-Neelsen staining in detecting low bacterial loads.

KEYWORDS: GeneXpert, NTEP, Pediatric Tuberculosis, Rifampicin Resistance, LPA

INTRODUCTION

Tuberculosis (TB) remains a significant public health threat globally, with an estimated 10.0 million cases diagnosed in 2020.[1] Children, particularly those under 15 years, constitute a substantial portion of the TB burden, accounting for approximately 12% of all cases.[2] This vulnerability is further compounded by the frequent occurrence of extra-pulmonary TB in this age group, which can manifest in various clinical presentations and pose significant diagnostic challenges.[3, 4]Traditional methods for diagnosing TB, such as sputum culture and microscopy, are often impractical in children due to the difficulty in obtaining adequate sputum samples.[5] Likewise, the Mantoux test, a valuable tool in adult TB diagnosis, has limited sensitivity in children, especially in malnourished individuals.[6] Chest X-rays, while occasionally helpful in older children, often lack specificity in younger age groups.[7]

Addressing these limitations is crucial for improving pediatric TB diagnosis and treatment outcomes. In recent years, the emergence of Cartridge-Based Nucleic Acid Amplification Tests (CBNAAT), like GeneXpert, has offered promising advancements. These rapid and accurate diagnostic tools can detect Mycobacterium tuberculosis complex DNA in various clinical specimens, including respiratory samples and even non-respiratory specimens like cerebrospinal fluid, within hours.[8, 9] Moreover, GeneXpert offers the additional benefit of simultaneously detecting rifampicin resistance, a critical factor in guiding treatment decisions.[10]Studies have

shown promising results for using GeneXpert in diagnosing childhood TB, with higher sensitivity compared to conventional methods. [11, 12] Additionally, the feasibility of using alternative specimens like buccal swabs in children with GeneXpert further enhances its potential in this population. [13] However, questions remain regarding the optimal role of GeneXpert in comparison to traditional methods like Line Probe Assay (LPA) and acid-fast staining in various clinical settings in India. [14, 15]

This study aims to evaluate the prevalence of TB and rifampicin resistance among pediatric patients in our setting. Furthermore, we will compare the diagnostic performance of GeneXpert with LPA and acid-fast staining to determine the most effective approach for diagnosing pediatric TB in our population. The findings from this study will contribute to a better understanding of the TB burden in children and inform optimal diagnostic strategies to improve early detection and treatment success in this vulnerable population.

MATERIAL AND METHODS

Study Design and Duration: This retrospective study was conducted over one year, from 1st January 2022 to 31st December 2022, at a tertiary care hospital in Western Maharashtra.

Inclusion and Exclusion Criteria: Samples from patients under 18 years of age were included. Inadequate Samples or samples from patients over 18 years were excluded.

Sample Collection and Testing: For sputum samples, early morning, deeply expectorated sputum samples were collected in sterile containers from clinically suspected TB cases aged 18 years or younger, with informed consent. For children unable to produce sputum, gastric lavage was collected, and other extrapulmonary samples were obtained as per the case.

Samples were tested using the GeneXpert MTB/RIF assay (Cepheid, France), a cartridge-based nucleic acid amplification technique (CBNAAT) that uses real-time PCR to detect Mycobacterium tuberculosis and rifampicin resistance by targeting the rpoB gene.

GeneXpert MTB/RIF Procedure: For each fresh sample, 2 ml of sample reagent was added to 1 ml of the sample in the collection container. After shaking vigorously and incubating at room temperature, the sample was processed until it was fluid and clump-free. A minimum of 2 ml of the processed sample was transferred into a GeneXpert cartridge and analyzed. Indeterminate results for rifampicin resistance were repeated using a second sample.

Microscopy and Culture: All samples underwent Ziehl-Neelsen staining with 25% H_2SO_4. CBNAAT-positive samples were further analyzed using the Mycobacterial Growth Indicator Tube (MGIT) culture and/or Line Probe Assay (LPA). For MGIT culture, a 100 μl portion of the deposit was inoculated into MGIT tubes containing PANTA antibiotics and growth supplements, and incubated in a MGIT 960 machine for up to 42 days. Positive cultures were tested for rifampicin susceptibility using a BACTEC MGIT SIRE kit (Becton, Dickinson).

Line Probe Assay (LPA): LPA was performed at the Intermediate Reference Laboratory in Aundh, Maharashtra, for the detection of isoniazid resistance and second-line drug resistance.

RESULTS

This study was conducted in a tertiary care hospital in Western Maharashtra, involving 528 samples of clinically suspected pediatric tuberculosis (TB) patients. The gender distribution showed a nearly equal split, with 49.43% males and 50.57% females (Table 1). The highest proportion of suspected cases was observed in the 0-5 years age group, accounting for 46.21% of the total cases.

Age (Years)	Male No.(%)	Female No.(%)	Total No.(%)
0-5	116 (21.97)	128 (24.24)	244 (46.21)
6-10	89 (16.86)	73 (13.82)	162 (30.68)
11-15	41 (7.76)	51 (9.66)	92 (17.42)
16-18	15 (2.84)	15 (2.84)	30 (5.68)
Total	261 (49.43)	267 (50.57)	528 (100)

Table 1: Age and Gender Distribution in Clinically Suspected TB Patients

Among the various sample types received for testing, gastric aspirate was the most common, comprising 81.25% of the total samples, with a positivity rate of 10.49%. Sputum samples had a positivity rate of 24.24%, while bronchoalveolar lavage (BAL) samples showed the highest positivity rate at 66.67%. Pus samples also had a positivity rate of 50%. Other extra-pulmonary samples such as cerebrospinal fluid (CSF) and pleural fluid exhibited lower positivity rates of 2.38% and 12.5%, respectively (Table 2).

Out of the 528 samples, 61 tested positive by CBNAAT, with a higher positivity rate in females (67.21%) compared to males (32.79%) (Table 3). The highest positivity rate was observed in the 11-15 years age group (34.43%), followed by the 16-18 years age group (32.79%).

Diagnostic yield of various modalities

Regarding the diagnostic yield, AFB staining was positive in 5.87% of the cases, while CBNAAT detected TB in 11.55% of the samples. Among the 26 samples tested by Line Probe Assay (LPA), 92.31% were positive, with 18 samples sensitive to both rifampicin and isoniazid, and 6 samples resistant to both. Of the 35 samples subjected to MGIT culture, 30.56%

Type of Sample	No. of Samples Received (%)	No. of Samples Positive (%)
Sputum	33 (6.25)	8 (24.24)
Gastric aspirate	429 (81.25)	45 (10.49)
Ascitic fluid	4 (0.76)	0 (00)
CSF	42 (7.95)	1 (2.38)
Pus	6 (1.14)	3 (50)
Pleural fluid	8 (1.51)	1 (12.5)
BAL	3 (0.57)	2 (66.67)
Biopsy material	2 (0.38)	0 (00)
Liver abscesses	1 (0.19)	1 (100)

Table 2: Sample Received for Testing in Pediatric Patients for TB

Age (Years)	Male No.(%)	Female No.(%)	Total No.(%)
0-5	6 (9.84)	7 (11.47)	13 (21.31)
6-10	3 (4.92)	4 (6.56)	7 (11.47)
11-15	5 (8.20)	16 (26.23)	21 (34.43)
16-18	6 (9.84)	14 (22.95)	20 (32.79)
Total	20 (32.79)	41 (67.21)	61 (100)

Table 3: Age and Gender Distribution in TB Positive Patients by CBNAAT

Variables	N (%)
AFB Staining	Tested - 528
	Negative - 497 (94.13%)
	Positive - 31 (5.87%)
CBNAAT	Tested - 528
	Negative - 467 (88.45%)
	Positive - 61 (11.55%)
LPA	Tested - 26
	Negative - 2 (7.69%)
	Positive - 24 (92.31%)
	Rifampicin Sensitive, Isoniazid Sensitive - 18
	Rifampicin Resistant, Isoniazid Resistant - 6
MGIT	Tested - 35
	No Growth - 23 (65.71%)
	Contamination - 3 (8.33%)
	Positive - 11 (30.56%)
Tested by Both MGIT and LPA	Tested - 11
	Only LPA Positive - 2
	Only MGIT Positive - 1
	Both MGIT and LPA Positive - 7
	Both MGIT and LPA Negative - 1

Table 4: Diagnostic Yield of Various Modalities

were positive, while 65.71% showed no growth. For the 11 samples tested by both MGIT and LPA, 7 were positive by both methods (Table 4).

DISCUSSION

This study was conducted over one year, focusing on pediatric patients under 18 years of age, who were clinically suspected of tuberculosis (TB). A total of 528 samples, including sputum, gastric lavage, cerebrospinal fluid (CSF), and other extrapulmonary samples, were collected and tested using GeneXpert (CBNAAT) and Acid-fast Bacillus (AFB) staining. Positive samples were further analyzed using either the Mycobacteria Growth Indicator Tube (MGIT) culture or Line Probe Assay (LPA), or both.

The majority of samples (81.25%) were gastric lavage, reflecting the difficulty in obtaining sputum samples from younger children, particularly those under 12 years of age. This correlates with previous studies, such as Singh et al., where the distribution of samples across different age groups and gender was comparable to our findings. [2] In our study, the highest number of samples (46.21%) was received from children aged 0-5 years, with a slight predominance of female patients (50.57%).

The overall positivity rate for TB in our study was 11.55%, aligning closely with the findings of William et al., who reported a similar positivity rate of 12% using CBNAAT. [10] This consistency underscores the reliability of CBNAAT as a diagnostic tool for pediatric TB. However, our positivity rate was lower than the 45% reported by Champatiray et al., likely due to their broader diagnostic criteria, which included clinical findings, neuroimaging, and various other diagnostic modalities. [9]

In terms of rifampicin resistance, our study found that 24.59% of the positive cases were rifampicin-resistant, a figure higher than the 9% and 12.75% reported in studies by Bhatia et al. and William et al., respectively. [10, 13] This discrepancy may be attributed to factors such as non-adherence to treatment protocols, inadequate isolation measures, and poor nutritional status, which are known to contribute to drug resistance. [16]

Interestingly, the 0-5 years age group, which accounted for the largest number of samples in our study, had a TB

positivity rate of 2.46%. This is significantly lower than the 12% prevalence observed in a previous study conducted at our institution in 2013, which focused exclusively on children under 5 years of age. [17] The decline in TB cases could be attributed to improved adherence to treatment protocols, better availability of medications, and more effective follow-up and counseling practices, including the testing of household contacts when a TB case is identified.

In terms of diagnostic efficacy, our study confirmed that CBNAAT is superior to AFB staining, with positivity rates of 11.55% and 5.87%, respectively. This difference is likely due to the higher sensitivity of CBNAAT, which can detect as few as 131 bacilli per milliliter, compared to the 5000-10000 bacilli per milliliter required for detection by ZN staining. [12, 15]

Among the 26 samples tested by LPA, 92.31% were positive, with a mix of rifampicin-sensitive and rifampicin-resistant cases. MGIT culture, although less sensitive, also provided valuable information, with a positivity rate of 30.56%. These findings are consistent with those of Bangarwa et al., who reported a sensitivity of 63.46% and specificity of 100% for LPA in detecting Mycobacterium tuberculosis. [14]

The variations in TB prevalence and diagnostic yields across different studies highlight the challenges in diagnosing and managing pediatric TB. Factors such as the type and quality of samples, regional differences in TB burden, and variations in diagnostic practices contribute to these discrepancies. Addressing these challenges requires ongoing efforts to improve diagnostic accuracy, adherence to treatment protocols, and follow-up care, particularly for vulnerable pediatric populations. [5, 12]

CONCLUSION

This study found a TB positivity rate of 11.55% among pediatric patients under 18 years of age, with rifampicin resistance detected in 24.59% of positive cases. The study highlighted the limitations of Ziehl-Neelsen staining, particularly in samples with very low or low bacterial loads, which were missed by this method but detected by CBNAAT. Notably, a significant decrease in TB positivity was observed in the 0-5 years age group over the past decade, suggesting that enhanced diagnostic accuracy, adherence to treatment protocols, improved nutritional awareness, and regular follow-ups at our tertiary care hospital have contributed to better outcomes. However, the persistence of drug-resistant TB underscores the urgent need for advanced diagnostic tests capable of detecting resistance to isoniazid and other first-line drugs, especially in cases where MGIT culture results are negative. Continued focus on these areas is essential to further reduce the burden of pediatric tuberculosis.

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REFERENCES

1. WHO Team Global Tuberculosis Programme (GTB). World Health Organization. Global Tuberculosis Report 2020; 2020. Available from: https://www.who.int/publications/i/item/9789240013131.
2. Singh MP, Sharma PK, Upadhyay P, Agrawal P, Gupta DK, Verma V. A cross sectional study of in-hospital cases of Pediatric Tuberculosis detected by CBNAAT at a tertiary care teaching hospital of Central India. Pediatric Rev Int J Pediatr Res. 2021;8(1):39–44.
3. Kruijshaar ME, Abubakar I. Increase in extrapulmonary tuberculosis in England and Wales. Thorax. 2009;64:1090–1095.
4. Ahmed T, Sobhan F, Ahmed AM, Banu S, Mahmood AM, Hyder KA. Childhood tuberculosis: a review of epidemiology, diagnosis and management. Infect Dis J Pakistan. 2008;17(2):52–60.
5. Datta S. Comparison of sputum collection methods for tuberculosis diagnosis: A systematic review and pairwise and network meta-analysis. The Lancet Global Health. 2017;5(8):30201–30203.
6. Kumar R, Gupta A, Aggarwal A. Diagnostic accuracy of tuberculin skin test in children with tuberculosis. Indian J Pediatr. 2010;77(1):15–18.
7. Singh M, Aggarwal A, Kumar R. Role of chest radiography in diagnosis of tuberculosis in children. Indian Pediatr. 2010;47(1):15–18.
8. Yadav RK. Cartridge based nucleic acid amplification test: Utility as diagnostic modality in clinically diagnosed childhood tuberculosis. Journal Of Clin And Diagn Res. 2020;.
9. Champatiray J, Patra GD. Diagnosis of paediatric tuberculosis by cartridge based nucleic acid amplification test and its effectiveness as compared to the other conventional diagnostic methods. Int J ContempPediatr. 2019;6:1204–1214.
10. William A, Rai Y, Kaur R. Evaluation of rifampicin-resistant tuberculosis in pediatric patients by GeneXpert MTB/Rif. Journal of Microbiology and Infectious Diseases. 2021;11(02):81–87.

11. Vasumathy R, Akalya R. Clinical profile of childhood tuberculosis and diagnostic efficacy of CBNAAT in tertiary care hospital. IAIM. 2020;7(11):23–28.

12. Raj A, Baliga S, Shenoy MS, Dhanashree B, Mithra PP, Nambiar SK et al. Validity of a CB-NAAT assay in diagnosing tuberculosis in comparison to culture: A study from an urban area of South India. J Clin Tuberc Other Mycobact Dis. 2020;21:100198–100198.

13. Bhatia R, Dayal R, Pipariya D. Detection of Mycobacterium tuberculosis in Buccal Swab Specimens in Children with Pulmonary Tuberculosis Using Cartridge-based Nucleic Acid Amplification Test. Pediatr Inf Dis. 2021;3(4):131–134.

14. Bangarwa M. Role of line probe assay in detection of mycobacterium tuberculosis in children with pulmonary tuberculosis. Indian Journal of Tuberculosis. 2023;p. 70–70.

15. Rasool G, Khan AM, Mohy-Ud-Din R, Riaz M. Detection of Mycobacterium tuberculosis in AFB smear-negative sputum specimens through MTB culture and GeneXpert® MTB/RIF assay. Int J Immunopathol-Pharmacol. 2019;33:2058738419827174–2058738419827174.

16. Jiang W, Fernandez M. Addressing the adherence challenge in tuberculosis treatment: More than digital technologies. Lancet Glob Health. 2023;11(5). doi:10.1016/S2214-109X(23)00160-2.

17. Jain SK, Ordonez A, Kinikar A, Gupte N, Thakar M, Mave V et al. Pediatric tuberculosis in young children in India: a prospective study. Biomed Res Int. 2013;p. 783698–783698.

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