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Research Article

GENEXPERT FOR GASTRIC LAVAGE TO DIAGNOSE PULMONARY TUBERCULOSIS IN CHILDREN

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Abstract- Rapid and accurate diagnosis of pulmonary tuberculosis in children is still a unique challenge because of difficulties in obtaining sputum samples and the paucibacillary nature of the disease. Objective of this study was to evaluate the utility of GeneXpert on Gastric lavage samples in children. This prospective study was conducted over a period of one and half year *i.e.*, from January 2016 to June 2017 in TB C & DST Laboratory, Department of Microbiology, Government Medical College, Aurangabad. During this period, 486 Gastric lavage samples of suspected Pediatric TB were referred to our laboratory. We processed by three methods which are microscopy, GeneXpert and Culture. Of the 486 GL samples, 5 (1.02%) were smear positive; 23 (4.73%) were GeneXpert positive and 7 (1.44%) were culture positive. In GeneXpert, one sample (4.34%) showed resistance to Rifampicin. In our study sensitivity of conventional methods in GL samples of children with pulmonary MTB was low as compared to GeneXpert. GeneXpert with advantages of quick turnaround time, simultaneously detecting rifampicin resistant TB provides a promising solution to the TB diagnostic challenges in children.

Key Word-: *Mycobacterium Tuberculosis*, Gastric Lavage, Ziehl-Neelsen staining, GeneXpert.

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Introduction

WHO Global Tuberculosis report 2016 states that TB remained one of the top 10 causes of death worldwide, approximately 1.2 million children become ill and 0.17 million died with it [1]. In India, daily up to 500 children die from TB and over three quarters of a million children fall ill with TB each year [2]. Diagnosis of pulmonary tuberculosis in children has relied predominantly on clinical, radiological, and tuberculin skin-test findings [3]. However, clinical diagnosis has low specificity, radiological interpretation is subject to inter-observer variability, and the tuberculin skin test is a marker of exposure, not disease [4]. Microbiological confirmation with identification of drug resistance is increasingly important in the context of an emerging drug resistant tuberculosis epidemic [5]. Early diagnosis is essential for early treatment initiation and to improve patient outcome. In developing countries, most TB control programmes uses ZN smear microscopy which is having low sensitivity leading to false-negative results and misdiagnosis of TB suspects. Also, this investigation requires multiple visits that leads to higher default [6]. Confirmation is delayed on culture. Thus, Conventional methods miss many cases of childhood tuberculosis [7]. For rapid detection and identification of *Mycobacterium tuberculosis* (MTB) in clinical specimens of pulmonary and extra-pulmonary tuberculosis cases, many Nucleic Acid Amplification (NAA) methods have been developed. For the diagnosis of TB, World Health Organization has approved the use of GeneXpert (Xpert® MTB/Rif assay) which utilizes a Real time DNA-PCR technique simultaneously detecting Rifampicin resistance related mutations providing results within 2 hours with a high diagnostic accuracy [8].

Aims and Objectives

1. To diagnose pulmonary pediatric tuberculosis
2. To diagnose cases of MDR TB in pediatric population
3. To evaluate the utility of GeneXpert, performed on GL in children

Material and Methods

Inclusion Criteria:

1. Gastric Lavage samples of suspected pulmonary tuberculosis patients of age ≤ 14 yrs [9].

Exclusion Criteria:

1. Patients with tuberculosis of age >14 yrs. 2. Patients already diagnosed with tuberculosis. 3. Rest all samples.

The study is a prospective over a period of one and half year *i.e.*, from January 2016 to June 2017 in TB C & DST Laboratory, Department of Microbiology, Government Medical College, Aurangabad. Paediatricians were asked to send gastric lavage samples under strict guidelines given to them with completely filled "requisition forms for pediatric tuberculosis" according to National pediatric guidelines [9]. 486 GL samples of suspected pediatric TB were referred to our laboratory. Before processing the samples, it is decontaminated by NALC- NaOH method then were subjected to three methods which are microscopy by Z-N staining, GeneXpert and Culture. GeneXpert testing was performed according to the manufacturer's instructions [10].

Results

Of the 486 specimens, 23 (4.73%) samples were positive and 463 (95.26%) specimens were negative by all three methods used [Table-1].

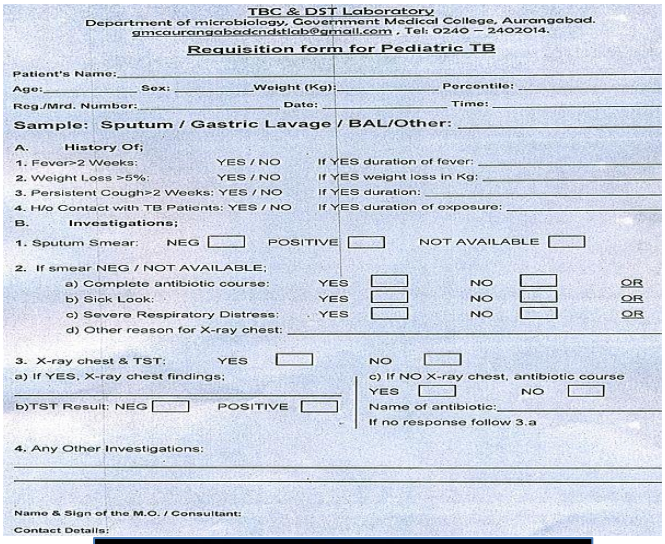
Table-1 *Positivity of MTB by all three methods*

Total samples tested	MTB positive cases	MTB negative cases
486	23(4.73%)	463(95.26%)

Microscopy; smear positivity was seen in 5 (1.02%) samples; rest 481 (98.97%) samples were smear negative [Table-2] [Fig-1].

Table-2 *Results of microscopy by Z-N staining of GL samples*

Total samples	Smear positive	Smear negative
486	5 (1.02%)	481 (98.97%)



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Requisition form for Pediatric TB

Patient's Name:
Age: Sex: Weight (Kg): Percentile:
Reg./Mrd. Number: Date: Time:
Sample: Sputum / Gastric Lavage / BAL/Other:

A. History Of;
1. Fever>2 Weeks: YES / NO If YES duration of fever:
2. Weight Loss >5%: YES / NO If YES weight loss in Kg:
3. Persistent Cough>2 Weeks: YES / NO If YES duration:
4. H/o Contact with TB Patients: YES / NO If YES duration of exposure:

B. Investigations;
1. Sputum Smear: NEG ☐ POSITIVE ☐ NOT AVAILABLE ☐

2. If smear NEG / NOT AVAILABLE;
a) Complete antibiotic course: YES ☐ NO ☐ OR
b) Sick Look: YES ☐ NO ☐ OR
c) Severe Respiratory Distress: YES ☐ NO ☐ OR
d) Other reason for X-ray chest:

3. X-ray chest & TST: YES ☐ NO ☐
a) If YES, X-ray chest findings;
b)TST Result: NEG ☐ POSITIVE ☐
c) If NO X-ray chest, antibiotic course
YES ☐ NO ☐
Name of antibiotic:
If no response follow 3.a

4. Any Other Investigations:

Name & Sign of the M.O. / Consultant:
Contact Details:

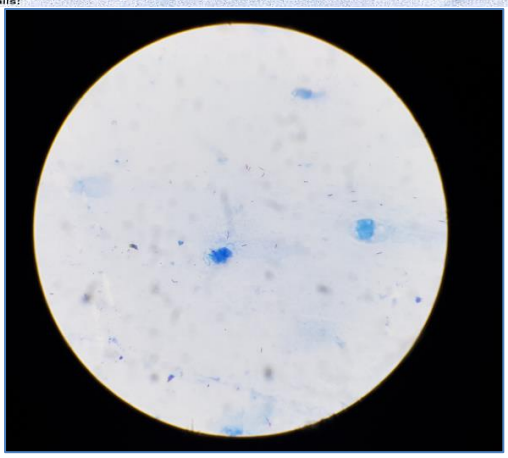

Fig-1 ZN stained Microscopy of *Mycobacterium tuberculosis*

GeneXpert detected 23 (4.73%) samples which were positive and in which one sample showed resistance to Rifampicin [Table-3].

Table-3 *Detection of MTB by GeneXpert of GL samples*

Total samples tested	MTB positive -23 (4.73%)		MTB negative
	MTB positive and Rifampicin sensitive	MTB positive and Rifampicin resistance	
486	22	1	463 (95.26%)

Of the 486 GL samples 7 (1.44%) were culture positive [Table-4] [Fig-2].

Table-4 *Culture positivity of GL samples*

Total samples tested	MTB positive cases in culture
486	7(1.44%)

Fig-2 Growth of *Mycobacterium tuberculosis* on LJ media from positive sample

Among 23 TB positive cases 18 (78.26%) were males and 5 (21.73%) were females. [Fig-3]. Maximum positivity was seen in 0-5 years of age group *i.e.*, 69.56% [Fig-4]. The youngest child diagnosed as a TB case was aged 5 months old and the oldest 14 yrs.

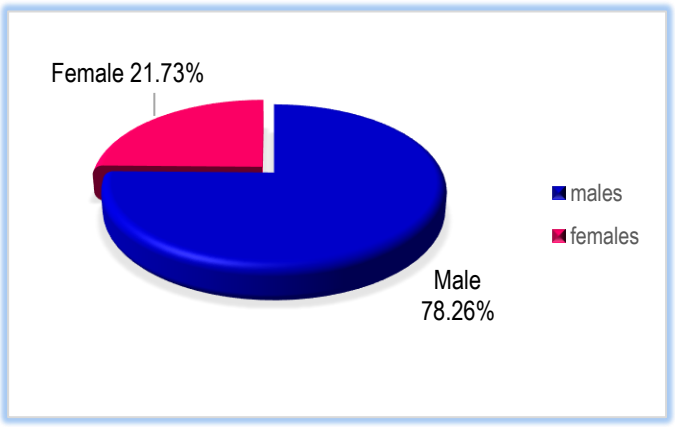

Fig-3 Sex wise distribution of TB positive cases in GL samples of pediatric patients

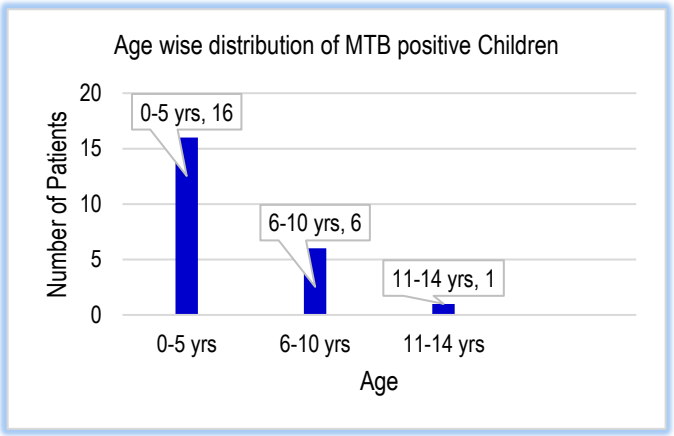

Fig-4 Age wise distribution of TB positive cases in GL samples of pediatric patients

Out of 23 positive patients, 3 (13.04%) died, 3 (13.04%) on treatment and remaining 17 (73.91%) completed treatment and are cured [Fig-5]. All three children died had fever < 2 wks, weight loss > 5 kgs, cough >2 wks, sick look and positive X ray finding. One was having history of contact with MTB positive patient.

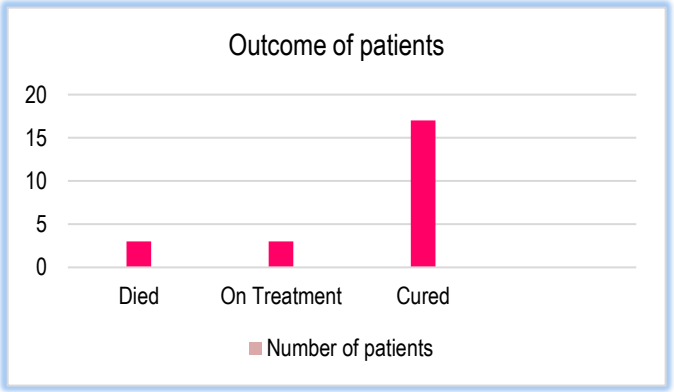

Fig-5 Outcome of patients

Discussion

TB samples are often paucibacillary and therefore it becomes very important to decide which sample is to taken to yield the highest sensitivity. The sensitivity of

GeneXpert is higher in sputum samples than gastric lavage but younger children can't expectorate sputum instead they swallow them, so there cannot be any better method other than Gastric Lavage to diagnose MTB in children [11].

In our study greater positivity was seen in age group 0-5 yrs *i.e.*, 69.56% as compared with age group 11-14 yrs *i.e.*, 4.34% in gastric lavage samples. Young children are more vulnerable for acquiring TB. Children from 1–2 years have a 20%–30% risk, those from 3–5 years a risk of 5%, those 5–10 years old only a 2% risk and older children an adult-like risk (5%).

We have also found that male predominance (78.26%) was noted in our study and male predominance was also seen in WHO Global Tuberculosis report 2016 which is 63%.

In this study we have evaluated the diagnostic yield of microcopy, GeneXpert and culture which is taken as gold standard.

Microscopy is primary method for detecting TB and monitoring treatment response. It is inexpensive and requires minimal biosafety standards. For reliably producing a positive result, smears require approximately 10,000 bacilli /mL [12]. This could explain why we got low positivity. The disadvantage of microscopy is that low bacterial load will give negative reporting, it provides no information on drug susceptibility of the bacilli, and it cannot distinguish between *Mycobacterium tuberculosis* complex and *non-tuberculosis mycobacteria* [12].

On the other hand, GeneXpert is the first fully automated CB-NAAT assay for MTB detection. One of the most important and obvious reason for the use of the GeneXpert is significantly reduced turnaround time for detection and it can detect approximately 150 viable or nonviable bacilli /ml. Not only is the turnaround time reduced to 2 h, this test can also detect rifampicin resistance simultaneously [12]. GeneXpert result can indicate that MTB was not detected or that MTB was detected and was not resistant to rifampicin or that MTB was detected and it was resistant to rifampicin [Table-5]. Gastric lavage on GeneXpert is having pooled sensitivity 66% (95% CrI, 51-81%) [13]. So, this explains that why we got 23 samples positive with GeneXpert despite very low positivity with microscopy.

Table-5 *Results from GeneXpert*

GeneXpert Results	No. of Patients
MTB detected very low; Rifampicin resistance not detected	8
MTB detected low; Rifampicin resistance not detected	6
MTB detected low; Rifampicin resistance detected	1
MTB detected medium; Rifampicin resistance not detected	7
MTB detected high; Rifampicin resistance not detected	1

But there are certain things which are to be taken care off like, a stable uninterruptable electrical supply is needed for operating GeneXpert. The ambient operating temperature cannot exceed 30 °C and cartridges must be stored at less than 28 °C. The shelf-life of the cartridges must be monitored to prevent them from expiring before they are used. In patients, who are living in low TB prevalence, tested positive for Rif resistance, a second GeneXpert test should be performed to control for preanalytical and postanalytical errors, and to improve the clinician's confidence in the diagnosis. Culture provides a definitive diagnosis of TB. Bacterial growth can be identified visually or by automated detection of its metabolism hence providing the necessary isolates for conventional DST. Culture is considered the reference standard as it can detect 10 -100 viable bacilli /ml. But results take weeks to obtain and testing requires a well- equipped laboratory and bio-safety precautions, highly trained staff, and an efficient transport system to ensure viable specimens [12]. We got 7 samples positive, mainly the reason could be improper and inefficient sample collection and transport mechanism as we need live bacilli to come culture positive. If we also refer to [Table-5]; 15 samples showed low or very low MTB detection, rest 8 samples showed medium or high detection on GeneXpert. Clinical features seen in MTB positive patients was also studied, maximum correlation seen with fever (91.30%) followed by sick look (86.95%), chest X ray (78.26%) and persistent cough (65.21%) [Table-6]. Bates M. et.al (2013) had similar results like ours in microscopy (1.14%) and in GeneXpert 3.55% which is slightly lower as compared to ours 4.73, but they have more culture positive cases comprising of 5.61% [14].

Table-6 *Showing correlation of clinical features with MTB positive Children*

Clinical Features	Co-relation with Positive samples
Fever < 2 wks	21 (91.30%)
Sick look	20 (86.95)%
Xray chest	18 (78.26)%
Persistent cough >2 wks	15 (65.21%)
Weight loss > 5 kgs	14 (60.86%)
H/O Contact	10 (43.47%)
Fever < 2 wks + Weight loss > 5 kgs + persistent cough	12 (52.17%)

Neeraj Raizada, *et al*., (2015) had microscopy results similar 1.5% to ours but have higher GeneXpert positivity 6% [15]. Whereas Duong TN. et.al (2015) have much higher positivity rate compared to us which is 6.51% in microscopy and 20.46% GeneXpert [16].

The use of Xpert MTB/RIF does not eliminate the need for conventional microscopy, culture and DST, which are required to monitor the progress of treatment and to detect resistance to anti-TB agents other than rifampicin [12].

Conclusion

Our study shows that GeneXpert is a useful in diagnosing pediatric tuberculosis rapidly and accurately. The Xpert MTB/RIF assay is significantly better than smear microscopy as far as GL samples are considered.

Application of Research

We detected 16 more MTB positive cases which were missed by conventional methods and they were treated successfully.

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Author's Contributions: All author equally contributed.

Abbreviations

MTB - Mycobacterium Tuberculosis
GL - Gastric Lavage
ZN - Ziehl-Neelsen
MDR TB - Multiple Drug Resistant Tuberculosis
Rif - Rifampicin
PCR - Polymerase chain reaction
CBNAAT - Cartridge based nucleic acid amplification test
NALC- NaOH method- N-acetyl L-cysteine- sodium hydroxide method

Conflict of interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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