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# COMPARISION OF PCR CATRIDGE BASED WITH ROUTINE PROCEDURE FOR DIAGNOSIS OF PAEDIATRIC TUBERCULOSIS & HUMAN IMMUNO DEFICIENCY VIRUS

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**Abstract Introduction:** Throughout ancient times, tuberculosis has posed a serious threat to humankind. Around 150 million years ago is when the Mycobacterium genus was first recognised. Egyptian mummies have been used to show the skeletal malformations caused by TB. Yet, the infectious agent behind this terrible sickness was not identified for quite some time.

**Material and Methods:** Between February 2022 and November 2022, the department of Microbiology at the Maheshwara Medical College and Hospital in Patancheru, Telangana conducted a prospective study. A total of one hundred people were included as part of this study's sample.

**Results:** Children who had expressed concerns were asked for and given permission to have a sample taken by their parents or legal guardians. Gastric aspirates, broncho-alveolar lavage, induced sputum, sputum, tracheal aspirate, ascitic fluid, lymph node aspirates, pleural fluid, and a synovial biopsy were all included in the collection of specimens. There was complete processing and analysis of all samples.

***Conclusion:*** This research was conducted to identify the most efficacious techniques for detecting TB in young people. CBNAAT has an advantage over other methods used to diagnose paediatric TB because it picked up more instances that were overlooked by those other approaches.

**Keywords**: Catridge, PCR, traditional techniques, diagnosing paediatric TB

## INTRODUCTION

TB has been a major threat to humanity for a very long time. Mycobacterium as a genus has been traced back to its beginnings about 150 million years ago. Egyptian mummies have been used to prove that TB causes skeletal abnormalities [1, 2]. But, for longer periods of time, the infectious agent behind this terrible sickness remained a mystery. Using light microscopy and a specialised staining technique, Robert Koch in 1882 discovered Mycobacterium tuberculosis, the bacterium responsible for TB [3, 4]. Lack of proper diagnostic procedures means that the prevalence of paediatric tuberculosis in underdeveloped countries like India is likely underestimated. Furthermore, difficulties in getting specimens and the amount of sample taken are typically scant in children, adding to the difficulty in detecting a case of paediatric TB [5, 6].

Microscopy has played a crucial role in the diagnosis of TB thus far since it is quick, cheap, and easy to use. Nevertheless, because paucibacillary strains of paediatric TB are below the 1010 threshold for detection by microscopy, their use in detecting a case is constrained. The use of fluorescent microscopes equipped with mercury vapour lamps or LED is restricted due to their higher cost and technical complexity [7, 8]. The gold standard for diagnosing TB is culturing Mycobacterium tuberculosis in solid or liquid media, although doing so needs highly specialised labs and trained personnel. It takes more time to cultivate a stable culture. Despite liquid cultures' quicker detection of M. tuberculosis, they are very vulnerable to contamination. Primary growth in the medium is required for drug susceptibility testing utilising these phenotypic approaches. Because of this, the process of identifying tuberculosis medication resistance takes even longer. Hence, this contributes to the spread of drug-resistant TB and a decrease in treatment efficacy [9-11].

The BACTEC 9000, Versa TREK, mycobacteria growth indicator tube, and microscopic observation drug susceptibility assay are some of the newer automated methods available for doing drug susceptibility testing. Yet, in TB-endemic countries, the quick diagnosis provided by nucleic acid amplification methods such as conventional and real-time polymerase chain reaction has not yet reached the people at the core level [12, 13].

Although several advanced techniques for TB diagnosis have been developed, a reliable point-of-care test is still necessary for the efficient management of TB in children. Although though TB is on the decline in India, the rise of TB subtypes such as MDR-TB, XDR-TB, and TB-HIV co-infection has slowed our progress towards an END-TB strategy [14, 15].

The use of cartridge-based nucleic acid amplification assays has been a great help in the rapid and accurate identification of tuberculosis, especially in instances involving children and in cases that have spread beyond the lungs. With a sensitivity of 131CFU/ml, the CBNAAT may be performed even on paucibacillary material. The CBNAAT not only identifies rifampicin resistance rapidly, but it also does so simultaneously. It is currently the standard method of initial analysis for both HIV-TB coinfection and extrapulmonary tuberculosis. Upcoming improvements to CBNAAT will allow for the development of near-patient diagnostic technologies that can detect TB at a reasonable cost [16, 17].
Our research seeks to better understand how cartridge-based PCR assays compare to more traditional approaches to diagnosing paediatric TB so that treatment can begin sooner and more effectively. The goal is to address diagnostic gaps and problems in children with TB.

## MATERIALS AND METHODS

Between February 2022 and November 2022, the Department of Microbiology at the Maheshwara Medical College and Hospital, Patancheru Telangana, India, conducted a prospective study. A total of one hundred people were included as part of this study's sample.
The Maheshwara Medical College and Hospital Ethics Committee gave their permission for the study to proceed. The study was approved by the administration of each hospital division involved. The author interviewed each and every one of the kids that participated. At the time of the interview, patients filled out a standard proforma and gave their informed consent to participate in the study. SPSS 16.0 was used to analyse the data. Chi-square tests were applied to categorical data to determine statistical significance.

### Inclusion Criteria

1. Children under the age of 18 who have had a persistent cough or fever for more than two weeks.
2. Children under the age of 18 who have lost their appetite or experienced an unexplained weight loss or gain during the last three months.

3. Little children who have previously come into touch with a TB case that is contagious.
4. Minors with severe superficial lymphadenopathy under the age of 18.
5. Children under the age of 18 who have significant respiratory distress and unusual X-ray shadows.
6. Little children exhibiting signs of failure to flourish.

**Exclusion Criteria**

1. Those older than 18 with TB symptoms.
2. Patients under the age of 18 who have TB and are receiving anti-tubercular medication.
3. Patients under the age of 18 who are failure, default, and recurrence cases.

At least four hours passed without the kid being fed. The first thing parents are told to do when their child gets up in the morning is to have them lie down on their backs. Sterile gloves were donned, and the child's distance from their nose to their stomach was measured before a nasogastric tube was slowly put through their nose and into their stomach. All of the stomach's contents are sucked out using a syringe. If no fluid was drawn from the stomach, then the tube was confirmed to be in the stomach, 5-10 ml of normal saline was put into it, and the same amount was drawn out and placed in a sterile wide mouth container. Sodium bicarbonate of the same volume was added to it. The next day, the exact same thing happened.

**RESULTS**

N=100 kids with TB-related suspicions who visited the paediatric OPD were enrolled. Children who had expressed concerns were asked for and given permission to have a sample taken by their parents or legal guardians. Gastric aspirates, broncho-alveolar lavage, induced sputum, sputum, tracheal aspirate, ascitic fluid, lymph node aspirates, pleural fluid, and a synovial biopsy were all included in the collection of specimens. There was complete processing and analysis of all samples.

**Table 1: Age breakdown of the study population**

| Sr. No. | Age in years | Patients=100 | Total no. of positives N=6 |
|---|---|---|---|
| 1. | 1 to 5 | 52 | 2 |
| 2. | 6 to 10 | 38 | 2 |
| 3. | 11 to 18 | 10 | 2 |

One hundred samples were taken from kids under the age of five, and three of them tested positive for TB. Only one of the youngsters aged 6-10 was positive. There were around n=6 teenagers sampled, and one of them tested positive for TB.

**Table 2: Study distribution by gender Population**

| Gender | Total Patients n=100 | Total no. of positives N=6 |
|---|---|---|
| Male | 60 | 4 |
| Female | 40 | 2 |

We observed that there was a 1.30:1 ratio of boys to girls among the kids who took part in our study. Two out of about samples taken from male children tested positive for TB, while three out of approximately samples taken from female children tested positive. A Chi-square test performed on data broken down by gender indicated no statistically significant difference in the prevalence of detection between boys and girls.

**Table 3: Methods for Classifying Specimens**

| Sr. No. | Specimen | Total Patients n=100 |
|---|---|---|
| 1. | Gastric aspirate | 50 |
| 2. | Broncho alveolar lavage | 20 |
| 3. | Induced Sputum | 5 |
| 4. | Sputum | 5 |
| 5. | Tracheal aspirate | 5 |
| 6. | Ascitic fluid | 6 |
| 7. | Lymph Node aspirate | 4 |
| 8. | Pleural fluid | 3 |
| 9. | Synovial biopsy | 2 |

The majority (n=100) of the samples were taken from the lungs. Most of them came from people aspirating their stomachs. 7.9% of all samples come from outside the lungs.

**Table 4: Prevalence of contact among kids who could have TB**

| H/O Contact | TB Positive | TB Negative |
|---|---|---|
| Present (2) | 2 | 0 |
| Absent (100) | 3 | 90 |
| Total (100) | 5 | 90 |

Two of the children who tested positive for TB had a history of contact with someone who had the disease, whereas three of the children who tested negative for tuberculosis did not.

**Table 5: Mantoux test results for children suspected of having tuberculosis**

| Mantoux test | TB Positive | TB Negative |
|---|---|---|
| Positive (8) | 4 | 4 |
| Negative (95) | 1 | 95 |
| Total (100) | 5 | 100 |

Eighty percent of TB-positive individuals and just 4.5% of TB-negative cases had positive results on the Mantoux test.

**Table 6:** The two most common types of staining, acid fast and fluorescent, are compared and contrasted

| | ZN Acid faststaining | KINYOUN Acid faststaining | Fluorescentstaining |
|---|---|---|---|
| **M.tb Positive** | 1 | 1 | 2 |
| **M.tb Negative** | 99 | 99 | 100 |

The fluorescent staining identified twice as many instances as the acid fast staining, which was only able to identify one.

## DISCUSSION

The incidence at which TB was found in children in our research was 4.3%. In the last five years, the number of cases reported of paediatric tuberculosis has risen from six percent to ten percent over the world. When compared with our research, the detection rates in countries like Zimbabwe, Uganda, South Africa, and Kenya were all quite comparable. Other nations, including Ethiopia, Afghanistan, and the Democratic Republic of the Congo, have higher detection rates. Compared to

other countries, Brazil, Thailand, Vietnam, Indonesia, Russia, China, Burma, and Bangladesh all had lower detection rates [18, 19].

The claims that poorer nations have a lower rate of notification are untrue. This is because not all instances are reported, therefore the true cost is likely substantially greater. Infants, toddlers, and young children are more vulnerable to contracting tuberculosis because of close proximity to an infected family member or close friend. There is a paucity of accurate information on the incidence of TB in children because of low clinical suspicion and a lack of adequate point-of-care tools for the diagnosis [20, 21].

In the case of paediatric TB, age is a critical aspect since it impacts the likelihood of the infection progressing to full-blown illness. When one's age lowers, so does the danger. The likelihood of infection is greatest in newborns and drops to 20%-30% in toddlers and young school-aged children. The risk is 5% for preschoolers and 2% for kids in elementary school. The risk rises to 5% once again in early adolescence [22].

According to our study's age-based analysis, the prevalence of TB was highest in children younger than 5 years old, and decreased in those younger than 15 years old. The United States of America also recorded quite high rates, with 41% of cases occurring between the ages of 5 and 15, and 59% occurring in children younger than 5 years old [23].

Nevertheless, there was a discrepancy between our study and a report from Europe, where the percentage of TB cases in children younger than 5 years old was 40% and in children younger than 15 years old it was 60%. In our study, we found no evidence of a gender difference in the identification of TB in children less than 5 or older than 15 years of age. Several investigations from different regions of the world showed consistent results. Detection rates vary with age because to hormonal changes throughout puberty and other reasons unique to men. Research shows that men have a greater rate of detection than women do [24].

Our research found that pulmonary TB accounted for 80% of cases and extrapulmonary tuberculosis accounted for 20%. These results are consistent with those of previously published research from the United States that found 75% of TB cases in the lungs and 25% in other organs among children and young adults under the age of fifteen. Researchers in the Middle East have found no difference in the detection rates between pulmonary and extra pulmonary TB [25].

Among the children in our research, pulmonary TB was the most common type in those less than five years old, whereas the percentage of pulmonary and extra pulmonary cases was similar among those older than five years old. To our knowledge, there is no correlation between the age distribution of a paediatric population and the ratio of pulmonary to extrapulmonary TB. Even in Turkey, researchers found no age-related differences in the prevalence of tuberculosis they were able to identify in their sample of young people [26].

As the immune system of teenagers is more resistive to tubercle bacilli, the extra pulmonary illness of TB is most prevalent in this age group. Children are more likely to develop extra-pulmonary TB as they become older because of the disease's tendency to reactivate and spread after first targeting the lungs. According to the results of these investigations, the pulmonary and extra pulmonary percentages in children less than five were 76% and 24%, respectively, but these figures dropped to 66% and 34% in children older than five [27].

Forty percent of the positive cases in our investigation had a previous exposure to an infected individual. Most kids get sick through interacting with infected adults, so knowing their history of

exposure is crucial. Among children less than five years old who were diagnosed with active TB, around 30% had previously been into touch with an infected case in their community, according to studies conducted in Pune and Chennai. With this information, healthcare providers can reduce the spread of disease among children and implement preventative measures. This means that a child's risk of contracting TB is precisely proportional to the number of people he or she has been into touch with in the past [28].

The Mantoux test is still one of the initial diagnostic steps taken in underdeveloped nations when paediatric TB is suspected. Although it can be used as a secondary piece of evidence in the diagnosis of paediatric TB, its applicability is restricted due to the fact that it may be negative in 10%-25% of instances where tuberculosis is actively present. Eighty percent of the tuberculosis-positive patients in our research tested positive on the Mantoux. Mantoux positive in various kinds of paediatric TB was found at 34.7% in a research conducted at the Institute of Child Health in Chennai, making this a very high number. Just two of the children who tested positive for tuberculosis in our research had a positive Mantoux result, most likely because of immuno suppression. Moreover, in several Mantoux-positive kids, we could not identify an infectious epicentre. Possible explanation: a bogus good reaction to the BCG vaccine [27, 28].
We obtained a detection rate of 1 for rifampicin-resistant M. tuberculosis among a total of 115 youngsters. According to one study conducted in Africa, 6.7% of TB-affected youngsters are resistant to treatment. The resistance rate in our study143 was comparable to the rates reported by other research, which ranged from 0.6% to 2.3% in paediatric TB. Because most children become infected through close contact with an infected adult, it is important to note the considerable disparity in the rates of resistance between adults and children. Possible causes include a lack of awareness of the condition and a consequent lack of reports of paediatric cases, as well as a lack of access to children's samples [29-30]. The study's low positive rate is an issue because of the small sample size. Results may improve if the study is expanded to include more participants or is carried out as a multi-centric study.

## CONCLUSION

This study was conducted to identify the most effective diagnostic strategies for identifying paediatric TB. CBNAAT has an advantage over other procedures since it could identify more instances of paediatric TB that would have been overlooked by more traditional methods. Of the confirmed cases, 0.9% were found to have TB strains resistant to rifampicin. A quick approach that can identify both M.TB and rifampicin resistance at the same time is necessary because of the rising trend of drug-resistant tuberculosis in youngsters. As a result, CBNAAT is the gold standard for diagnosing TB in children. If TB is detected and treated early enough, it can be stopped from spreading across a community, greatly reducing the number of child deaths from the disease.

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