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PROFILE OF EXTRA-PULMONARY TUBERCULOSIS IN PEDIATRIC PATIENTS: A STUDY FROM A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Extra-pulmonary tuberculosis (EPTB) involves sites other than the lungs. Diagnosing EPTB poses significant challenges due to the need for invasive procedures, specialized expertise, the paucibacillary nature of samples, and the presence of non-specific signs and symptoms. The main objective of this study was to determine the occurrence of EPTB in the pediatric age group, compare diagnostic methods such as Ziehl-Neelsen staining, Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), and Mycobacterial Growth Indicator Tube (MGIT) culture method, and evaluate rifampicin resistance in pediatric patients. **Material And Methods:** This prospective observational study was conducted over one and a half years after obtaining permission from the institutional ethics committee. All clinically suspected extra-pulmonary samples for tuberculosis received from patients under 18 years of age, except blood, stool and urine. Samples were collected and processed according to National Tuberculosis Elimination Program (NTEP) guidelines and subjected to Ziehl-Neelsen staining, CBNAAT, and MGIT culture. **Results:** A total of 303 clinically suspected EPTB samples were received, with lymph node and pus samples constituting the majority. **Out of the total samples tested, 71 (23.43%) were positive on CBNAAT;** while MGIT detected 63 (20.79%), and the smear examination identified only two (0.66%). Rifampicin resistance was observed in 4 of the 71 positive samples (5.6%). **Conclusion:** CBNAAT remained the primary diagnostic tool for EPTB due to its rapid results and ability to detect rifampicin resistance. Pediatric EPTB is yet a major health concern since it might be underdiagnosed in India due to limited clinical suspicion.

KEYWORDS : CBNAAT, EPTB, MGIT, NTEP, Pediatric.

INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis*, primarily targets individuals with compromised immune systems. When it involves areas of the body beyond the lungs, such as lymph nodes, meninges, kidneys, spine, or bone growth plates, it is classified as extra-pulmonary tuberculosis (EPTB). Globally, EPTB accounts for approximately 20–30% of all tuberculosis (TB) cases. Indian studies identify lymph node TB as the most common form of EPTB. Diagnosing EPTB is particularly challenging as it is seldom smear-positive.

Ziehl-Neelsen staining rarely yields positive results in EPTB cases with low bacterial loads, while culture methods are time-consuming. CBNAAT emerged as an essential tool for diagnosing EPTB. This study analyzed EPTB cases over one and a half years in a tertiary care hospital to achieve the following objectives:

1. Study the occurrence of *M. tuberculosis* in extra-pulmonary samples from pediatric patients.
2. Determine the prevalence of rifampicin resistance.

MATERIAL AND METHODS

This prospective observational study was conducted in the Department of Microbiology at a tertiary care centre over a period of 18 months. Before conducting the study, approval was obtained from the institutional ethics committee. The study included children upto 18 years of age associated with EPTB, while patients above 18 years of age, blood, stool, BAL, gastric lavage, nasal aspirate and urine samples were excluded.

Samples were processed following NTEP and Central Tuberculosis Division guidelines and subjected to microscopy by Ziehl Neelsen staining, CBNAAT (GeneXpert MTB/RIF, Gen IV, Cepheid, Sunnyvale, USA) and BACTEC MGIT 960 system (Becton Dickinson and Company, Maryland, USA

RESULTS-

Considering the age distribution, the maximum clinically suspected samples were seen in the age group of 11-18 years (68.97%) followed by 6-10 years group (17.49%), and last by 1-5 years group (12.21%). Clinically suspected females were more as compared to males (Females- 62.37%, males-37.63%).

A total of 303 extra-pulmonary samples were received for the diagnosis of *M. tuberculosis*. The maximum samples received were those of lymph node aspirate 95 (31.35%), followed by CSF 48 (15.84%) and pus 47 (15.51%). The least number of samples received were of pericardial fluid and synovial fluid. (Table I)

Amongst extra-pulmonary samples positive for *M.tuberculosis*, maximum were lymph node aspirates (24) followed by pus (21) on CBNAAT. (Table II)

All 303 samples were tested by Ziehl-Neelsen staining, CBNAAT and MGIT. 71 samples were found positive by CBNAAT, 59 by MGIT and only 2 by Ziehl-Neelsen staining. (Table II). Sensitivity and Specificity of CBNAAT (100%) in our study. (Table III)

Out of 71 positive samples, 4 (5.63%) samples were rifampicin-resistant. (Table IV)

Tables

Table I: Clinically Suspected Extra-pulmonary Samples Received From Pediatric Patients-

Type Of Samples	Total Number Of Samples Received	Percentage (%)
CSF	48	15.84
FNAC	35	11.55
Lymph node	95	31.35
Pericardial fluid	1	0.33
Peritoneal fluid	6	1.98
Pleural fluid	41	13.53

Pus	47	15.51
Synovial fluid	1	0.33
Tissue	29	9.57
Total	303	100

Table II: Number Of Extrapulmonary Samples Positive By Ziehl Neelsen Staining, CBNAAT And MGIT

Type of samples	Positive by Ziehl Neelsen staining	Positive by CBNAAT	Positive by MGIT
CSF	0	7	6
FNAC	0	8	7
Lymph node	2	24	22
Pericardial fluid	0	0	0
Peritoneal fluid	0	0	0
Pleural fluid	0	4	3
Pus	0	22	17
Synovial fluid	0	0	0
Tissue	0	6	4
Total	2 (0.66%)	71 (23.43%)	59 (19.47%)

Table III: Sensitivity And Specificity Of CBNAAT (GeneXpert MTB/RIF, Gen IV, Cepheid, Sunnyvale, USA)

Type of samples	Sensitivity (100%)	Specificity (100%)
CSF	100	100
FNAC	80	100
Lymph node	100	100
Pericardial fluid	Not calculable	100
Peritoneal fluid	Not calculable	100
Pleural fluid	100	100
Pus	100	100
Synovial fluid	Not calculable	100
Tissue	100	100

Table IV: Rifampicin Resistance In Pediatric Extra-pulmonary Samples By CBNAAT

Rifampicin Resistance	Number of samples	Percentage (%)
Detected	4	5.63
Not detected	67	94.37
Total	71	100

DISCUSSION-

This study was conducted at a tertiary care hospital for a duration of one and a half years. Extra-pulmonary samples in clinically suspected patients less than 18 years of age were included in the study.

Extra-pulmonary tuberculosis (TB) is more common in children aged 0-10 years due to several factors. One of the main reasons is that their immune systems are not fully developed, making them more susceptible to TB infections affecting organs other than lungs.[5]

Additionally, the symptoms of extra-pulmonary TB in children can be less specific or non-specific, making the diagnosis more challenging and often leading to delayed treatment. Also, malnutrition may weaken the immune system which may lead to TB.[6]

In this study, clinically suspected females were more as compared to males (Females- 62.37%, males- 37.63%). In a study conducted by Aurilio RB et al, De Oliveira MC et al and Kaba Ö et al, 51.2%,47.2 and 47.1% participants were females respectively.[5-7]The greater number of female participants in our study could be due to health of females being ignored as compared to males. Also, hormonal and physiological factors may contribute to the same.

In our study, out of 303 extra pulmonary sample analyzed, maximum were lymph node 95 (31.35%), followed by CSF 48 (15.84%) and pus 47 (15.51%), FNAC 35 (11.55%), pleural fluid 41 (13.53) tissue 29 (9.57%) nasal aspiration 27 (8.54%), peritoneal fluid 6 (1.98%), pericardial fluid 1 (0.33%), synovial fluid 1 (0.33%). Tortoli E et al reported that most samples, total 368 (25%), were tissue biopsies or fine-needle aspirates, followed by pus samples 195 (13.2%), cerebrospinal fluid (CSF) 133 (9%), and urine samples 130 (8.8%).[8]

Among the 71 positive samples 2 (2.81%) smears were positive for acid fast bacilli on ZN stain, 59 (83.09%) samples grew *Mycobacterium Tuberculosis* using Liquid culture MGIT 960 system, and CBNAAT detected 71 (100%) samples. In a study conducted by Piersimoni C et al in which 11 smear and culture were positive for *M. tuberculosis* & 29 samples were positive by CBNAAT.[9] Smear microscopy is a traditional method of diagnosing TB. However, its sensitivity is limited by the number of bacteria present in the sample, which can be low in the case of extra-pulmonary TB (World Health Organization, 2019).[10] As a result, *Mycobacterium tuberculosis* in these samples may not be present in sufficient numbers (limit of detection-10000 CFU/ml) to be visible under a microscope, leading to false negative results. In addition, smear microscopy can be affected by the quality of the sample, the staining method used, and the experience of the microbiologist interpreting the results.[10] In contrast, CBNAAT is a molecular diagnostic test that uses real-time polymerase chain reaction (PCR) to detect the presence of *Mycobacterium tuberculosis*. CBNAAT has high sensitivity and can detect *Mycobacterium tuberculosis* even when the number of bacteria present is low (limit of detection-131 CFU/ml), making it a highly sensitive and specific diagnostic tool for TB.[10] MGIT is a culture-based system that can rapidly grow and detect *Mycobacterium tuberculosis* in samples, including those from extra-pulmonary sites. Its ability to grow and detect *Mycobacterium tuberculosis* in a sample, even when the number of bacteria present is low (limit of detection, 10-100 CFU/ml), makes it a more effective diagnostic tool for extra-pulmonary TB compared to smear microscopy.[11]

A study by Tortoli E et al showed CBNAAT was positive in 188 samples in which 71 (37.76%) biopsy samples were positive for *M. tuberculosis,* followed by pus 40 (21.28%), CSF 11 (5.85%), pleural fluid 5 (2.66%) and cavitary fluid 5 (2.66%).[8] Causse M et al, showed CBNAAT was positive in 41 samples in which 18 (43.90%) biopsy samples were positive for *M.tuberculosis,* followed by CSF 6 (14.63%), purulent exudates 5 (12.19%) & pleural fluid 4 (9.76%).[12] In a study conducted by Vadwai et al., 98 pus samples (34.63%) tested positive for *M. tuberculosis*, followed by body fluids, with 24 samples (8.48%) showing positivity.[13]

The sensitivity of the MGIT culture method is known to be lower compared to CBNAAT in detecting EPTB for several reasons. Firstly, MGIT is a culture-based method, which requires the growth of *M. tuberculosis*, the bacterium that causes tuberculosis, in a culture medium. This can be a slow process, taking several weeks to obtain a result. During this time, the bacterial culture can become contaminated, or the bacteria can die, reducing the sensitivity of the test. In contrast, CBNAAT is a rapid molecular test that provides results within a few hours of obtaining a sample. Secondly, EPTB samples may contain low numbers of mycobacteria, making it difficult to obtain a positive result using the MGIT culture method. In such cases, CBNAAT may provide a more sensitive result because it is able to detect the genetic material of *M. tuberculosis* directly in a sample, without the need for culturing the bacteria. Lastly, CBNAAT has the added advantage of being able to simultaneously detect resistance to rifampicin, an important first-line drug for tuberculosis treatment. This information can help guide treatment decisions and is not available with the MGIT culture method.[14-16]

Rifampicin resistance was identified in 4 out of 71 extrapulmonary samples (5.63%) using CBNAAT. These findings align with the study by Avashia S et al from Indore, which reported rifampicin resistance in 5.4% (6/111) of cases through CBNAAT. Similarly, a study by Gupta S et al found

rifampicin resistance in 5.8% (5/85) of extrapulmonary samples, further corroborating our results.[18]

CONCLUSION-

Diagnosis should not rely solely on smear examination, as the detection rate of *M. tuberculosis* is low, and MGIT culture requires considerable time. CBNAAT provides rapid results and facilitates early detection of rifampicin resistance. In our study, 23.43% of cases were positive for *M. tuberculosis* on CBNAAT, with 5.63% showing rifampicin resistance, emphasizing the crucial role of molecular testing in ensuring timely and effective TB management. Therefore, CBNAAT serves as a game changer not only in the control of pulmonary tuberculosis but also in extrapulmonary tuberculosis, especially in pediatric EPTB cases, which might be underdiagnosed in India due to limited clinical suspicion in the pediatric population.

Conflict Of Interest- None

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