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COMPARATIVE ANALYSIS OF CONVENTIONAL METHODS WITH CATRIDGE BASED PCR METHOD IN THE DIAGNOSIS OF PAEDIATRIC TUBERCULOSIS

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ABSTRACT: Background: Tuberculosis is a great menace to mankind since ancient times. The genus mycobacterium was known to originate 150 million years ago. Despite the discovery of many newer methods in the diagnosis of tuberculosis, a robust point of care test is required for effective management of Paediatric tuberculosis. **Methodology:** We conducted this study in paediatrics department of N.M.C.H for 1 year. After obtaining written informed consent, we enrolled cases in our study. All cases were subjected for focussed history from Guardians. Gastric Aspirate, Sputum, Induced Sputum, Ascitic fluid, CSF were collected is a wide mouthed container for sputum microscopy & in Falcons tube for CBNAAT. The microscopic examination of the samples were done by LED microscope using Z-N staining Technique. **Results:** Out of 51 samples analysed by microscopy and CBNAAT, CBNAAT has higher positivity rate (17.6%) than microscopy technique (5.8%). Result analysed using chi square test, considering CBNAAT as gold standard. The resulting value (p=0.027) interprets that the p value is less than 0.05. It is statistically significant. **Conclusion:** This work was done to find out ideal diagnostic methods for diagnosis of tuberculosis among children. Among the methods which were used for the diagnosis of paediatric tuberculosis CBNAAT was advantageous.

INTRODUCTION: Tuberculosis is a great menace to mankind since ancient times. The genus mycobacterium was known to originate 150 million years ago. The discovery of mycobacterium tuberculosis, the causative agent for tuberculosis was by Robert Koch in 1882 using light microscopy and a special staining technique. India accounts for above 2 million cases every year since 2018, India has world‘s highest share of all Tb cases.

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According to estimates, childhood Tb constitutes 10-20% of all Tb cases in high burden countries like India. Drug resistance strains of Mycobacterium Tuberculosis are also highly prevalent among paediatric population [1]. In India, about 3,42,000 new cases of paediatric TB are estimated to occur every year accounting for 31% of the global burden and 13% of overall TB burden.

Although pediatric TB is estimated to be approximately 10% of total incident cases, only 5.6% of total cases reported to the National TB Elimination Programme (NTEP) are children and this proportion is almost similar over last several years [2]. Despite the discovery of many newer methods in the diagnosis of tuberculosis, a robust point of care test is required for effective management of Paediatric tuberculosis.

Though Tuberculosis shows a decreasing trend in India, tuberculosis in children and adolescents. MDR TB, XDR TB, TB-HIV co-infection are increasing at an alarming rate and have hindered our progress towards an END-TB strategy.

The aim of our study is to compare the cartridge based PCR assay (CBNAAT) with the conventional methods for a better and earlier diagnosis of paediatric tuberculosis. The purpose is to fill the lacunae and resolve the difficulties in the diagnosis of paediatric tuberculosis.

MATERIAL AND METHODS:

Study Participants: Children of age group < 18 yrs admitted in department of Paediatrics, Nalanda medical College and Hospital, Patna in time period of 1 yr.

Study Period: AUGUST 2023-JULY 2024

Study Design: Prospective study

Inclusional Criteria:

1. Children < 18 years of age with symptoms of Koch‘s bacilli infection.
2. Children <18 years of age who has history of contact with an infectious TB case.
3. Children <18 years of age with significant superficial lymphadenopathy.

Exclusion Criteria:

1. Children > 18 years of age with symptoms of tuberculosis.
2. Children < 18 years of age who is a known case of tuberculosis and on anti-tubercular treatment.
3. Children < 18 years of age who are relapse, default and failure cases.

Study Technique: After obtaining written informed consent, we enrolled cases in our study. All cases were subjected for focussed history from Guardians. Gastric Aspirate, Sputum, Induced Sputum, Ascitic fluid, CSF were collected is a wide mouthed container for sputum microscopy & in Falcons tube for CBNAAT. The microscopic examination of the samples were done by LED microscope using Z-N staining Technique.

Statistical Analysis: The data was tabulated in Microsoft excel spread sheet in a master chart and studied for correlation. Statistical analysis of the data was conducted & the sensitivity, specificity PPV, NPV was calculated for AFB smear and CBNAAT as the gold standard. Data analysis was done by SPSS software version 29.0.

OBSERVATION AND RESULT: Around (n=51) children suspected of tuberculosis who were admitted are included in the study. Samples were collected from children based on their complaints after obtaining their parent‘s or guardian‘s consent for the same.

TABLE 1: TYPE OF SPECIMEN

Type of specimen	Total (51)
Gastric aspirate	26
sputum	16
Induced sputum	3
Pleural fluid	3
Ascitic fluid	1
CSF	1
Lymphnode Aspirate	1

Among the specimens collected gastric aspirates (51%) were predominant followed by sputum (31.4%). The extra pulmonary samples (n=6) constitute 11.7% of the total samples collected. Ascitic fluid, CSF and lymph node aspirate were among least collected samples.

43.2% Samples were collected from 0-5 yrs age group which was highest (n=22), followed by 6-10 yrs age group (37.2%). 10 samples we recollected from age group 11-18 yrs. Predominant sample type in lower age group was Gastric aspirate while sputum was predominant in higher age groups.

TABLE 2: PATIENTS AMONG DIFFERENT AGE GROUPS

	0-5 yrs	6-10 yrs	11-18 yrs
total patients	22	19	10

Among total, Males were predominant in our study constituting 27 samples out of 51 approx 53% of all.

TABLE 3: RESULT ON MICROSCOPY

Result on microscopy	Positive	Negative
Total	3	48
%	5.8	94.2

3 Out of 51 samples were positive on microscopy. (5.8%)

TABLE 4: AGE WISE RESULT ON MICROSCOPY

Age wise result on microscopy	Positive	Negative
0-5 Yrs	0	22
6-10 Yrs	2	17
11-18 Yrs	1	9

Among AFB+ve population 6-10 yrs age group has more positivity than other age groups and females having more positive rate in comparision to males.

TABLE 5: RESULT ON CBNAAT

Result on CBNAAT	Positive	Negative
Total	9	42
%	17.6	82.3

Among 51 samples which were analysed by CBNAAT method, 9 samples were positive for acid fast bacilli which constitutes about 17.6 percent of total analysed samples.

TABLE 6: AGE WISE RESULT ON CBNAAT

Age wise result on CBNAAT	Positive	Negative
0-5 Yrs	2	20
6-10 Yrs	4	15
11-18 Yrs	3	7

Positivity rate among 11-18 yrs age group was higher (42.8%), followed by 6-10 yrs group (26.7%). with increasing age positivity rate has also increased.

TABLE 7: MICROSCOPY VS CBNAAT

Zn staining	CBNAAT	
Mtb+	3	9
Mtb-	48	42
%	5.80 %	17.60%

Out of 51 samples analysed by microscopy and CBNAAT, CBNAAT has higher positivity rate (17.6 %) than microscopy technique (5.8%).

TABLE 8: MICROSCOPY VS CBNAAT (AGE WISE)

Age Group	Positive on microscopy	Positive on CBNAAT
0-5 YRS (n=22)	0	2
6-10 YRS (n=19)	2	4
11-18 YRS (n=10)	1	3

In younger age group (0-5 yrs) Ziehl-Neelsen staining technique fail to detect AFB bacilli while, CBNAAT was positive among 2 cases. In every age group, positivity rate by CBNAAT method was higher.

Result analysed using chi square test, considering CBNAAT as gold standard. The resulting value (p=0.027) interprets that the p value is less than 0.05.

It is statistically significant. Considering CBNAAT as gold standard, sensitivity, specificity, PPV and NPV was calculated.

TABLE 9:

Microscopy	CBNAAT+	CBNAAT-	Total
Positive	3(TP)	0(FP)	9(TP+FP)
Negative	6(FN)	42(TN)	42(FN+TN)
Total	9(TP+FN)	42(FP+TN)	51(TP+FN+FP+TN)

The formulas below were used to calculate the microscopy sensitivity and specificity:

Sensitivity =100 (True positive) / (True positive + false negative)

The diagnostic sensitivity of microscopy was 33.3%.

Specificity =100 (True negative) / (True negative + false positive)

The diagnostic specificity of microscopy was 100 %. Positive predictive value is calculated as TP/(TP+FP). Positive predictive value of the microscopy was found to be 100%. Negative predictive value of a test is calculated as TN/ (FN+ TN). Negative predictive value of the microscopy was found to be 87.5%. While considering Ziehl-neelsen staining technique as gold standard, sensitivity, specificity, PPV and NPV was calculated. Sensitivity of CBNAAT is 100% and specificity is 87.5%. NPV of CBNAAT is 100% and PPV is 33.3%.

TABLE 10:

CBNAAT	Microscopy+	Microscopy-	Total
Positive	3	6	9
Negative	0	42	42
Total	3	48	51

DISCUSSION: In our study the detection rate of tuberculosis was 17.6 %. Positivity rate has been seen higher in few studies but most of the studies have similar positivity rate.

In study of Rohon Das *et al* positivity rate was 36% [3]. In a study by Sameera *et al* positivity rate was 96% [4]. The difference in positivity rate may be due to different sample size and variation in different types of specimens. In our study, sensitivity of microscopy was 33.3% and CBNAAT was 100%. Most of the studies have shown similar sensitivity by microscopy method. In other study in 2020, sensitivity of CBNAAT was 84.43% [5]. Manish *et al in* 2023, microscopy showed positivity in 4 cases (3.92%) while CBNAAT on 18 cases out of 102 children [6]. A study by Dipti *et al* showed sensitivity by microscopy to 37.5 % and by CBNAAT to 75% [7]. Hanumanth Raju *et al* in a study showed sensitivity by microscopy to 7.5% [8]. In our study, on comparing the CBNAAT in detecting positive cases of tuberculosis with microscopy, we found that the sensitivity of CBNAAT was far better than microscopy (33.3%). Most of the studies have shown higher sensitivity of CBNAAT than microscopy.

CONCLUSION: This work was done to find out ideal diagnostic methods for diagnosis of tuberculosis among children. Among the methods which were used for the diagnosis of paediatric tuberculosis CBNAAT was advantageous as it could detect more cases which are missed by other conventional methods.

Limitations: The limitation of the study is low positivity rate due to very less sample size. If the extended to a wider sample size or is conducted as a multi-centric study, it may give better results. Follow up of cases could not be done. This was essential in cases of the Xpert positive, culture negative patients to know if they were responding to ATT and thereby to establish if these cases were actually "true positives"

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CONFLICT OF INTEREST: None

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