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Rapid Molecular Detection Ofmycobacterium Tuberculosis and Rifampicin Resistanceamong Pediatric Patients in a Tertiary Care Hospital of Northeast India

Majumder Nilratan[1], Reang Anita S[2], Sutradhar Balaram[3], Das Banti[4], MajumderTapan[5], Datta Shib Sekhar[6]

1 *Professor, Department of Paediatrics, Index Medical College, Hospital and Research Centre, Indore*
2 *Assistant Professor, Department of Paediatrics, Karpaga Vinayaga Institute of Medical Sciences & Research Centre*
3 *Research Assistant, Department of Paediatrics, Agartala Government Medical College, Tripura*
4 *Research Assistant, Department of Microbiology, Agartala Government Medical College, Tripura*
5 *Professor, Department of Microbiology, Agartala Government Medical College, Tripura*
6 *Professor, Department of Community Medicine, Tripura Medical College & Dr. BRAM Teaching Hospital*

Abstract
Introduction: In the era of rapid diagnosis of tuberculosis (TB) and drug resistance, Cartridge Based Nucleic Acid Amplification Technique (CBNAAT) came with a future of early and easy diagnosis for TB. However, microscopic examination of sputum sample remains most commonly used screening test and Lowenstein-Jensen (LJ) culture method is considered as gold standard. **Aim:***To diagnose tuberculosis among pediatric age group by CBNAAT and compare the results obtained with conventional methods.* ***Materials and method:*** *Present cross-sectional study was conducted during December 2016 to November 2018 among 120 consecutively selected children with signs and symptoms of TB. Detailed socio-demographic history, clinical examination was done using a pre-tested interview schedule. Tuberculin test was performed and sample was collected following standard protocol.* The performance of CBNAAT including *resistance to Rifampicin*and smear microscopy among samples obtained from paediatric patients was compared.***Results:***Among 120 pediatric suspects,majority (61.7%) were in the age groups of 0-5 years. Only one child was confirmed for TB by initial sputum microscopy. 12 (10%) children were positive for TB by both LJ culture and CBNAAT, one was found indeterminate by both these methods; remaining were negative for TB.Out of 12 cases found positive for TB, 11 (91.7%) were sensitive against Rifampicin and only 1 (8.3%) was resistant. Sensitivity, specificity, positive and negative predictive value of acid-fast staining taking LJ culture as reference standard was 33.3%, 100%, 100% and 93.3% respectively. Sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT were all 100%.* ***Conclusion:*** *CBNAAT showed 100% sensitivity, specificity, positive predictive value and negative predictive value in comparison to LJ media in our study. Gene expert assay is more efficient compared to smear microscopy for early diagnosis of suspected pulmonary M. tuberculosis with gastric aspirate sample in pediatric patients.*

Keywords: Drug resistance, Gastric aspirate, Paediatric tuberculosis

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I. Introduction

Tuberculosis (TB)is one of the major global health problems and rank next only to HIV as the leading cause of death from an infectious disease worldwide. According to World Health Organization(2016), there were 10.4 million new TBcases and 1.8 million TB deaths globally.[1]As per the End-TB strategy endorsed by 194 member states, the target set was to reduce TB deaths by 90% and 80% reduction of TB incidence by 2030 taking 2015 as the base year.[2] According to the Global TB report 2017, TB burden in India was approximately 2.8 million accounting for quarter of worlds TB cases.[2]As per Global TB report 2016, in 2015, 1 million children (0-14 years) fell ill with TB (10% of the total case load).[1] Early detection and effective treatment of infectious TB and multi-drug-resistant TB (MDR-TB) is the way forward to reduce the burden of childhood TB and child MDR-TB.[3]The diagnosis of tuberculosis in children is complicated due to absence of practical gold standard diagnostic test. Sputum microscopy has an important role in diagnosis of tuberculosisin adults but its usage in detection of pediatric*M.tuberculosis* is limited as they are paucibacillary and lower limit of detection using microscopy is 10,000.[4]

When Cartridge Based Nucleic Acid Amplification Technique (CBNAAT)was compared with other diagnostic techniques like smear microscopy andLowenstein-Jensen (LJ) culture; CBNAAT proved to be nearly accurate and matched the standard culture method, considered as gold standard.[5-7]TheCBNAAT besides providing faster results also detects resistance to Rifampicin simultaneously.[6] It is also being used as a first line of investigation in HIV-TB co infection and extra pulmonary TB.

The aim of this study was to diagnose tuberculosis among pediatric age group by CBNAAT and compare the results obtained with conventional methods.

II. Materials And Method

The present cross-sectional study was conducted at Agartala Government Medical College and Govind Ballabh Pant Hospital, Agartala between December, 2016 to November, 2018.

Children (less than 12 years) with sign and symptom suggestive of TB (with or without history of contact with TB patient in past 2 years, significant superficial lymphadenopathy, severe respiratory distress, abnormal shadows in skiagram or failure to thrive) were included.Children above 12 years of age, already diagnosed with TB or admitted with life threatening condition were excluded.

Children coming to the department of Pediatrics were first assessed for sign and symptoms of Tuberculosis (fever, cough for more than 2 weeks) and also assessed for any risk factors like history of contact with a known case of TB, MDR-TB or with person who died of TB/failed treatment of TB. Then patients' guardians were asked to give informed written consent on his /her behalf to be included in this study. The consent was sought in modified ICMR template in local language which participants can understand. Patients were assured about the confidentiality and anonymity during the whole study procedure and also informed that they can withdraw their given consent at any time of their convenience. Participant fulfilling inclusion criteria and also given informed written consent were enrolled in the study. Selected consecutive patient till desired sample size (calculated to be 120) were enrolled in the study.

The socio-demographic characteristics, detailed history and meticulous clinical examination was done on every patient and noted in a pre-designed and pre-tested interview schedule. Followed, tuberculin test was performed; sample was collected and sent to Department of Microbiology, under strict precaution in a sterile container.

Gastric aspirate, sputum, induced sputum, broncho-alveolar lavage, ascetic fluid, pleural fluid, lymph node aspirate was collected and processed following standard protocol.A standard method was followed for preparing smears of sterile samples (pleural fluid, ascetic fluid and lymph node aspirate).

CBNAAT was performed according to manufacturer instruction maintaining all aseptic precaution. CBNAAT is a real time polymerase chain reaction (PCR) which utilizes molecular beacon technology for detection of Rifampicin resistance. Molecular beacons are nucleic acid probes that specifically bind to "wild type" sequence of rpoB gene, Rifampicin susceptible gene. When the probe binds to such a sequence, a fluorescent signal is lighted up indicating the presence of Rifampicin susceptible TB. If the probe fails to bind, then it is considered as Rifampicin resistant TB.

III. Results

Among 120 study subjects,74 (61.7%)were in 0-5 years, 30 (25%) in 6 -10 years, and 16 (13.3%) in 11-12 years age group respectively. Gender-wise, 62 (51.7%) and 58 (48.3%) were male and female children**(Table1)**. Almost equal number of children represented rural and urban areas and equal proportion of children belonged to middle and lower socio-economic group, and only 0.8% belonged to upper class.

Total 72 (60%)children presented with fever,76 (63.3%) presented with cough more than 2 weeks and 21 (17.5%)children had history of contact with TB or MDR-TB cases. 40 (33.3%) children had characteristics chest features on X-ray suggestive of TB.

Gastric aspirate sample were collected from all 120 and sputum from 48 (40%) children. Extra pulmonary samples like pleural fluid, cerebrospinal fluid (CSF), ascetic fluid and FNAC from cervical swelling were also collected**(Table2)**. Only 1 child was confirmed for TB by initial sputum microscopy. Total 12 (10%)children were positive for TB by both culture and CBNAAT, and only 1 (0.8%) case was found indeterminate by solid LJ media culture as well as CBNAAT; rest 107 (89.2%) were negative for TB.**(Table3)**. Out of 12 cases found positive for TB, 11 (91.6%) were sensitive for Rifampicin, and only 1 (8.3%) was resistance against Rifampicin.

Sensitivity, specificity, positive and negative predictive value of acid-fast bacilli (AFB) staining taking LJ culture as reference standard was 33.3%, 100%, 100% and 93.3% respectively; as against sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CBNAAT all being 100%.**(Table 4 and 5)**.

Table1:Distribution of study children according to age and gender

	Frequency (n=120) N (%)	**Positives (n=12) N (%)**	**p value**
Age (in years)			
0-5	74 (61.7)	8 (10.8)	0.80 (NS)
6-10	30 (25)	2 (6.6)	
11-12	16 (13.3)	2 (6.6)	
Gender			
Male	62 (51.7)	7 (5.83)	0.66 (NS)
Female	58 (48.3)	5 (4.17)	

Table 2:Samples collected from study children during the study (n=120)

Sample	**Frequency (%)**
Pulmonary suspects	
Gastric aspirate	120 (100)
Sputum	48 (40)
Extra-Pulmonary suspects	
Pleural fluid	2 (1.7)
CSF	3 (2.5)
Ascitic fluid	1 (0.8)
FNAC	1 (0.8)
Total	**175**

Table: 3. Confirmed cases of TB with solid culture and CBNAAT (n =120)

	Frequency (%)		**Frequency (%)**
CST		**CBNAAT**	
Positive	12 (10)	Positive	12 (10)
Indeterminate	1 (0.8)	Indeterminate	1 (0.8)
Negative	107 (89.2)	Negative	107 (89.2)

Table: 4. Sensitivity, specificity, PPV and NPV of AFB staining against culture as reference standard

AFB staining	**Culture**		**Total**
	Positive N (%)	Negative N (%)	
Positive	4 (100)	-	4 (100)
Negative	8 (6.9%)	108 (93.1)	116 (100)
Total	12 (10)	108 (90)	120 (100)

(Sensitivity 33.3%, Specificity 100%, PPV100%, NPV 93.3%)

Table 5:Sensitivity, Specificity, PPV and NPV of CBNAAT against culture as reference standard

CBNAAT	**Culture**		**Total**
	Positive N (%)	Negative N (%)	
Positive	12 (100)	-	12 (100)
Negative	-	108 (100)	108 (100)
Total	12 (10)	108 (90)	120 (100)

(Sensitivity, Specificity, PPV, NPVall 100%)

IV. Discussion

World Health Organization has reported that children represent 10% of all TB cases. Zimbabwe, Uganda, South Africa, Kenya reported 3-4% of detection rate; whereas, higher rate i.e. 5-8% was observed in Ethiopia, Afghanistan, Democratic Republic of Congo, and lower rate i.e.1-3% has been observed in Brazil, Thailand, Vietnam, Indonesia, China, Myanmar and Bangladesh.[8]

Age is an important factor in the diagnosis of pediatric TB. The risk increases as the age decreases. Rate of infection is highest among the infants (50%), whereas in children of age 1-2 years it decreases to 20-30%. The risk among the pre-school children (3-5 years) is 5%, which further declines in case of primary school children (5-9 years).[9]In the present study, it was observed that majority of the study children were in the age group of 0-5 years (61.7%), followed by 6-10 years (25%) and 11-12 years (13.3%) respectively. Abinaya et al., (2018),[10] in their study also found similar results.However, our results differ with the findings of Kumar et al.,(2018),[11]where they observed that 47.6% children were in the age group of less than 5 years, 43% were in the age group of 5-9 years, and 9.4% were in the age group of 10-12 years. This difference may be attributed to under reporting of cases, lack of ideal point of care-technologies, and low clinical suspicion.

Spread of TB is closely linked to poverty and low socio-economic status.[12]This is further influenced by overcrowding and poor health-care awareness in low socio-economic environment.[13]Both these features are

present in the study area across study participants and this might have acted as a confounder in the present study.

The CBNAAT gives quicker result and requires minimal bio-safety requirements as well as minimal training for laboratory staff. CBNAAT is also of greater clinical utility as it has greater AUC (Area Under the ROC Curve is 99.02).[6]We also observed that, out of 19 Montoux positive cases, only 5 (41.7%, 5 out of actual 12 cases) were Mycobacterium tuberculosis positive when tested with CBNAAT. Whereas, of the total 101 Montoux negative cases, 7 were confirmed *Mycobacterium tuberculosis* positive when tested with CBNAAT. Our resultsare in line with findings of Kumar et al., (2018).[11] The probable reasons for better performance of CBNAAT can be explained by the above stated facts.

Chest Skiagram abnormalities may be suggestive of TB; however, it is not a confirmation of TB; although chest radiographs may be used to rule out the possibility of pulmonary TB. In the present study, we observed that 33.3% children had characteristic features on X-ray suggestive of TB.

Table 6: Comparison of sensitivity and specificity of various studies on CBNAAT and culture

Name	Year	Sample	Sensitivity	Specificity
WHO	2013	Pooled study Respiratory sample	66	98
Bates et al., Zambia	2013	Respiratory sample (Sputum)	90.8	99.3
		Gastric aspirate	62	98
Detjen et al., Germany	2015	Induced sputum	62	98
		Gastric aspirate	66	98
Md. Tanvir Ahmed, Bangladesh	2016	145 with Sputum sample	100	100
Present study, Tripura, India	2016-18	120 with Gastric aspirate	100	100

We observed that analysis of gastric aspirate samples yielded higher rate of *Mycobacterium tuberculosis*cases with CBNAAT in comparison to AFB staining. This confirms our hypothesis as CBNAAT having higher sensitivity and specificity. We also observed that all smear positive specimens were positive in Expert MTB/RIF assay, which indicates 100% concordance in smear positive case detection by Expert MTB/RIF assay. Ahmed et al. also got similar results in their study conducted in Bangladesh (2016).Study by Bunyasi et al., (2015)obtained similar results with gastric lavage.[14]In their study, of the 486 gastric lavage samples, 5 (1.02%) were smear positive; 23 (4.73%) were Gene Expert positive and 7 (1.44%) were culture positive.

In our study,only one patient was detected as Rif resistance out of total 12 positive cases. Detjen et al., (2015) reported expert pooled sensitivity and specificity to detect Rifampicin resistance was 62-66% and 98% respectively.[15]Total 16 published studies by WHO we reviewed revealed average sensitivity of Expert MTB/RIF to detect Rifampicin resistance in specimen from children was 86%.

V. Conclusion

The comparison of CBNAAT against LJ media in our study shows sensitivity, specificity, PPV and NPV of CBNAAT to be 100%. Gene Expert MTB/RIF assay (CBNAAT) is more efficient compared to smear microscopy for early diagnosis of suspected pulmonary tuberculosis with gastric aspirate in pediatric patients.

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