RESEARCH ARTICLE

MOLECULAR DIAGNOSIS BY CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (XPERT MTB /RIF ASSAY) - AN EARLY DIAGNOSIS AND BREAKTHROUGH IN INITIATION OF TREATMENT IN CHILDHOOD TB MENINGITIS AND PRIMARY MDR CASES

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ABSTRACT

Background: Tuberculosis (TB) still remains the major public health threat in India. Early diagnosis, so as to initiate early treatment is a priority as any delay, may complicate the prognosis further leading to the failure of an effective control. India accounts for 6% incidence of pediatric TB cases in a population that has 40% as estimated latent TB cases. Pediatric samples as the key population. Tuberculosis remains the commonest cause of childhood meningitis in high burden countries (Raj *et al.,* 2014). India accounted for highest number of TB deaths among children in the year 2015 followed by Nigeria and China. Out of which TBM stands to be the most common cause of morbidity. Extra pulmonary TB accounts for 25% out of the total TB cases reported among which the most severe form being the TBM. Though severe and difficult to diagnose due to lack of specific tests to get confirmed, CBNAAT is the only solution for microbiological confirmation of the disease for early treatment initiation and reduction of morbidity and mortality. Previously, due to lower case detection rates owing to inadequate diagnostic facilities of higher analytical sensitivity the mortality and morbidity continued to be in the higher range and empirical treatment modality was the main stay till the Cartridge based Nucleic acid amplification tests came into existence. The increase in the case detection for Tubercular meningitis at a manageable stage of the disease would help in decreasing the mortality rates. The study explores the increased case detection rates among pediatric population suffering from tubercular meningitis from referral units to Intermediate Reference Lab Kolkata. CSF from the pediatric patients has not been very difficult procedure though invasive specially in the pediatric age group and can be practiced at peripheral unit having CBNAAT facility. **Methods:** Cerebrospinal fluid from pediatric patients showing obvious clinical signs of meningitis were collected from tertiary unit linked to Intermediate Reference Laboratory Kolkata and tested on CBNAAT by Xpert *Mycobacterium tuberculosis* Rifampicin (MTB/RIF) (Cepheid) along with all the pulmonary and extra pulmonary samples based on the manufacturer's instruction. The data were captured in the Xpert software automatically during the tests and exported to the Microsoft Excel sheets for further analysis. A defined study design against each and every objective was set up to analyze the effective detection of tubercular meningitis case and their resistance to Rifampicin along with the other pulmonary and extra pulmonary samples. **Result and Conclusions:** The study concluded that confirmation of tubercular meningitis cases by the help of CBNAAT. The detection of microbiologically confirmed tuberculosis by these specimen sent from the referral unit linked with IRL Kolkata for pulmonary and extra pulmonary cases along with tubercular meningitis cases is significant and facilitate the early initiation of treatment for tuberculosis and more so to those who are newly detected as MDR TB especially below the 6 months of age. About 5% prevalence and around 4.9% incidence is an alarming situation in the TB control scenario of West Bengal. The positive case detection among the CSF samples was found to be more in the 0.6 to one year group followed by the age group of one to five years. The percentage positivity of CSF is recorded as 2.38% and the percentage of Rifampicin resistance among positive cases from CSF was found to be 13.79% (n=28).

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INTRODUCTION

It is estimated that India accounts for 25% of global tuberculosis (TB) burden of the world. The pediatric population accounted for 6% of the total burden (TB India 2017). In West Bengal, India, before the availability of cartridge based nucleic acid amplification test (CBNAAT), the pediatric TB was seen in 3.8% cases among the total population tested (Mukherjee *et al.,* 2014). As the initial diagnosis was solely dependent on smear microscopy, paucibacillary samples were left undetected. It is estimated that India accounts for around 40% of the latent TB infection (Mahmood, 2016). The pediatric case detections were less owing to a dearth of a sensitive technology not being able to detect the underlying cause (Raj *et al.,* 2014; Chakravorty *et al.,* 2005; Raizada *et al.,* 2015). The drug resistance cannot be detected by smear microscopy. Nucleic acid amplification methods are able to detect *Mycobacterium tuberculosis* complex (MTBC) categorically along with the point mutations

at the rifampicin (RIF) resistance determining region (Rufai *et al.,* 2014). Moreover the analytical sensitivity of smear microscopy is not sufficient enough as it accounts for a poor-positive predictive value owing to its nonspecific detections (Desikan, 2013). Among the extra pulmonary TB cases which contributes to 25% of the total case load, the most severe form was seen in Tubercular meningitis cases. The following study explored the increased case detection for the pediatric group showing clinical signs of meningitis after the availability of CBNAAT at the districts of West Bengal. The alarming data of more than 4% TB Pediatric TB prompted in further collection of samples. The detection of tuberculosis in pediatric age group is a difficult task either pulmonary or extra pulmonary as the sample collection is critical in these cases and disease like tubercular meningitis in these age group where the only specimen for confirmatory microbiological diagnosis is cerebrospinal fluid, though difficult but can even be done at the peripheral units with minimum expertise to collect but specific for diagnosis and life saving too which helps in early initiation of treatment.

METHODS

The objectives included the most common referral sites sending samples to Intermediate Reference Laboratory, Kolkata, for the suspected cases of tuberculosis meningitis of pediatric age group. The cerebrospinal fluid is subjected to Cartridge Based Nucleic Acid Amplification Test for microbiological confirmation.

Sample selection: Cerebrospinal fluid for all the suspected tubercular meningitis cases among the pediatric age groups was tested, irrespective of their geographical location that came to the Intermediate Reference Laboratory with a request for CBNAAT have been included in the study.

Study Design: The methods involve prospective analysis of the CBNAATs data of the pediatric samples coming to Intermediate Reference Laboratory, Kolkata, from May 2014 to May 2017. The proposal was reviewed and approved by Research and Ethical committee Intermediate Reference Laboratory, Kolkata India. The Ethics Committee Approval number STDC/IRL – CBNAAT Peadiatrc project no 012/ 2014. dated 5th Jan 2014 -2017. CSF samples were collected by the Pediatricians at the block level /tertiary care hospitals and aseptically transferred to IRL Kolkata. Data were captured for the entire period from the recording and registration register, the study designed was worked out based on the available data using computational software. Selective screening helped in tracking the contribution of the referral sites. The age and sample related propensity in the case detection were also studied.

The study comprised not only the detection of MTBC by CBNAAT but also status RIF resistance if detected. A concordance was made with the specimen by its smear microscopy.Before the processing of the sample for Gene Xpert around 20 μl was taken for making smears on grease-free slides. These slides were stained and examined under a fluorescent microscope. The samples were processed as per the standard operating procedures led down by the Gene Xpert MTB/RIF guidelines. For Xpert, around 2–5 ml of sample was taken. In case of cerebrospinal fluid (CSF), the test was run with at least of 1 ml of sample. The samples were mixed with

same amount of sample diluent solution as provided by the manufacturer. The sample along with the buffer solution was made to stand for 15-20 minutes with intermittent gentle shaking in between for effective mixing. The tests were then run on CBNAAT machines as per the manufacturer's user manual (XPERT MTB/RIF User's Manual, 2018).

Statistical analysis: Data were captured on Microsoft Excel worksheets and age and sample-wise propensity results were expressed in terms of percentage. A Student's *t*-test was done between the paired data of the quarterly point, the prevalence showing $P < 0.05$ was obtained. Odds ratio between the smear results and CBNAAT positivity was calculated out using MedCalc Software (https://www.medcalc.org/calc/odds_ratio. php) (Medical Software, 2010).

RESULTS

Cerebrospinal fluid contributed to 8.68 % of the pediatric samples in the study. Among the total number of pediatric cases that came for TB testing extra pulmonary cases accounted for 16 %. Out of which Paediatric samples tested for TB meningitis was 53.3% of the total extrapulmonary cases. The positivity within this group was 2.38%. When seen in terms absolute figures it was 29 pediatric patient showing positivity in CSF out of 1223 tested cases of meningitis (percentage positivity of 2.38%). Among wihich Rifampicin resistant was found to be 13.7% among the tested positives.

Chart 1. Percentage contribution of the individual samples to the entire load of samples received (n=14,088)

	Percentage	Positivity
Bone marrow	0.007	0
Cervical aspirate	0.021	0
CSF	8.68	2.37
ET secretion	0.13	0
FNAB	0.028	50
Gastric aspirate	14.51	2.64
Gastric lavage	16.8	3.4
Induced sputum	6.5	2.49
Lymph node	1.36	32.29
Omentum	0.007	0
Pericardial fluid	0.15	4.54
Peritoneal fluid	0.056	0
Pleural fluid	2.51	1.97
Pus	0.78	35.45
Right colonic ulceration	0.01	50
Sputum	46.6	10.04
Synovial fluid	0.035	0
Tissue	0.12	0
Tracheal aspirate	0.085	0
Urine	0.021	0
Abcess	0.04	100
Ascitic fluid	0.37	0
BAL	0.88	10.4

CSF: Cerebrospinal fluid, ET: Endotracheal aspirate, FNAC: Fine needle aspirations cytology, BAL: Bronchoalveolar lavage

Table 1. Percentage detection in different age groups

Age	Percentage positivity
<0.5 years (6 months)	2.78
0.5-1 years	10.42
1-5 years	29.51
>5 years	57.27

Table 2. The age wise contribution toward positive case detection by cartridge‑based nucleic acid amplification tests

	CSF	Gastric aspirate	Gastric lavage	Induced sputum	Lymph node	Pleural fluid	Pus	Sputum	BAL
<0.6	2	6	10	2	1	0	2	3	1
0.6-1 years	12	23	36	9	3	0	7	9	2
1-5 years	10	19	24	11	27	3	13	176	3
>5 years	5	6	11	1	31	4	17	473	7

CSF: Cerebrospinal fluid, BAL: Bronchoalveolar lavage

Table 3. Sample type contribution toward *Mycobacterium tuberculosis* detection and Rifampicin resistance detection

	Samples	Positivity	RIF resistance	Percentage positivity	RIF-resistant positivity
Bone marrow	1	0	0	0	0
Cervical aspirate	3	0	0	0	0
CSF	1223	29	4	2.37	13.79
ET secretion	19	0	0	0	0
FNAC	4	2	0	50	0
Gastric aspirate	2045	54	2	2.64	3.7
Gastric lavage	2380	81	9	3.4	11.1
Induced sputum	923	23	5	2.49	21.73
Lymph node	92	27	11	29.34	40.74
Omentum	1	0	0	0	0
Pericardial fluid	22	1	0	4.54	0
Peritoneal fluid	8	0	0	0	0
Pleural fluid	355	7	1	1.97	14.28
Pus	110	39	8	35.45	20.51
Right colonic ulceration	2	1	1	50	100
Sputum	6678	661	87	9.89	13.16
Synovial fluid	5	0	0	0	0
Tissue	18	0	0	0	0
Tracheal aspirate	12	0	0	0	0
Urine	3	0	0	0	0
Abcess	6	6	0	100	0
Ascitic fluid	53	0	0	0	
BAL	125	13	0	10.4	0
Total	14,088	979	128	6.94	13.07

RIF: Rifampicin, MTB: Mycobacterium tuberculosis, CSF: Cerebrospinal fluid, ET: Endotracheal aspirate, FNAC: Fine needle aspirations cytology, BAL: Bronchoalveolar lavage

DISCUSSION

Among the top ten causes of death worldwide, TB accounts for one of the most important factors; approximately, 1.2 million children became ill and 0.17 million died with it (World Health Organization, 2016). Five-hundred children deaths due to TB and over three quarters of a million children fall ill with TB each year. The study was on multidimensional approach of pulmonary and extra pulmonary samples of pediatric age group where the test was microbiologically confirmed by CBNAAT. Among the all sample received 1233 were cerebrospinal fluid in clinically suspected cases of meningitis. As the suspected case of tubercular meningitis were diagnosed by the supportive or ancillary tests available and treatment was based on high index of clinical suspicion prior to the introduction of CBNAAT which enhanced the microbiologically confirmed disease and a concrete evidence prior to initiation of treatment. The percentage positivity among this group was found to be 2.38 percentage (n=1233) which is quite suggestive that this population remained unaddressed earlier prior to introduction of CBNAAT owing to dearth of adequate technology. Smear Microscopy, biochemical test and cytology has remained the sole diagnostic support in the block level facilities earlier but CBNAAT has been introduced at the peripheral level which increases the microbiologically confirmed tubercular meningitis in this era of evidence based medicine.

Conclusion

The tubercular meningitis cases were microbiologically confirmed by the help of Cartridge Based Nucleic Acid Amplification Test.

The specimen sent from the referral unit linked with IRL Kolkata for pulmonary and extra pulmonary cases along with clinically suspected cases of tubercular meningitis cases. The yield of the test are significant which facilitate the early initiation of treatment of tuberculosis and more so to those who are newly detected as MDR TB as primary case specially below the 6 months of age. The positive case detection among the CSF samples was found to be more in the 0.6 to one year group followed by the age group of one to five years. The percentage positivity of CSF is 2.38% and the percentage of Rifampicin resistance among positive cases from CSF was found to be 13.79% (n=28).

Conflicts of interest: There are no conflicts of interest.

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