International Journal of Contemporary Pediatrics
Bala S et al. Int J Contemp Pediatr. 2019 Jul;6(4):1588-1591
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20192760

Original Research Article

Study on cartridge based nucleic acid amplification test in children with Neurotuberculosis at RNT Medical College, Udaipur, Rajasthan, India

Shashi Bala, Suresh Goyal*

Department of Pediatrics, RNT Medical College, Udaipur, Rajasthan, India

Received: 28 March 2019
Accepted: 02 May 2019

***Correspondence:**
Dr. Suresh Goyal,
E-mail: sbapna.1@gmail.com

ABSTRACT

Background: The aim was to determine utility of Cartridge based nucleic acid amplification test (CBNAAT) in diagnosis of mycobacterium tuberculosis in children with neurotuberculosis diagnosed on the basis of clinical evaluation, CSF findings and neuroimaging.
Methods: A prospective randomized controlled trial was conducted in Pediatric Department of RNT Medical College, Udaipur, Rajasthan, India from July 2017 to June 2018. Total 110 children of age group of 6 months to 18 years with the diagnosis of tubercular meningoencephalitis (TBME) on the basis of clinical evaluation, CSF examination and neuroimaging were included in the study.
Results: A total 110 children were enrolled. Maximum number of cases admitted with TBME were among 1-5 years of age group (60.91%). CSF and gastric aspirate were examined by CBNAAT for MTB. 5 (4.55%) children had CBNAAT positivity in CSF. Gastric aspirate was positive among 16 (14.55%) children. None of the patient had CBNAAT positive result both in CSF and gastric aspirate.
Conclusions: TBME is a major health problem in children below 5 years. Gene Xpert assay has the potential to significantly improve and escalate the diagnosis of smear-negative body fluid specimens. CBNAAT for mycobacterium tuberculosis was positive in 5 (4.55%) children from CSF and 16 (14.55%) from gastric aspirate. Negative CBNAAT should not prevent any patient with suspected features of TBME from starting anti tubercular treatment (ATT) as sensitivity of this test remains low. Final judgement to start ATT should be based on clinical, biochemical and radiological profile especially in CNS tuberculosis.

Keywords: CBNAAT, Cererospinal fluid, Gastric aspirate, Tubercular meningoencephalitis

INTRODUCTION

Tuberculosis has existed for millennia and remains a major global health problem. Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). India has one of the highest burden of tuberculosis (TB) globally, accounting for around 20% of all new TB cases annually.[1] It is estimated that childhood TB constitutes 10-20% of all TB cases in high burden countries.[2] Approximately, 25% of pediatric TB cases are extrapulmonary, with tuberculous meningitis (TBM) being the most severe form.[1]

CNS tuberculosis is usually paucibacillary in nature and meningitis are more often because of immune mechanism rather than directly because of mycobacterium. Tuberculosis affecting other sites known as extra-pulmonary TB is rarely smear-positive. Most patient of tubercular meningoencephalitis diagnosed on the basis of

clinical evaluation, CSF findings and neuroimaging. Approximately a third of patients die soon after presenting to hospital and many of those surviving is left with severe neurological sequelae.[3,4] Early diagnosis and treatment for TBME have been shown in numerous studies to be the best predictor of survival.[5,6]

The Gene Xpert MTB/RIF (Cartridge Based Nucleic Acid Amplification Test-CBNAAT) is an automated real time polymerase chain reaction (PCR) assay designed for the rapid and simultaneous detection of mycobacterium tuberculosis and rifampicin resistance within 2 hours.[3,7,8]

METHODS

A prospective randomized controlled trial was conducted in Pediatric Department of RNT Medical college, Udaipur, Rajasthan, India from July 2017 to June 2018.

Study population

Total 110 children between 6 months to 18 years of age group admitted with a clinical diagnosis of tubercular meningoencephalitis were enrolled in the study.

Inclusion Criteria

Children between 6 months to 18 years of age admitted in Balchikitsalaya, MBGH, RNT Medical College, Udaipur, Rajasthan, India with diagnosis of tubercular meningoencephalitis on the basis of clinical evaluation (fever, headache, stiffneck, seizures, abdominal symptoms such as nausea, vomiting) with or without signs of meningeal irritation and CSF finding (moderate lymphocytic pleocytosis, moderately elevated protein levels, low glucose) with or without favourable finding on cranial imaging (hydrocephalus, basalmeningeal enhancement, tuberculoma, vasculitis leading to infarcts) and parents giving written informed consent.

Exclusion Criteria

Age less than 6 months and parents not consenting for participation in study.

Procedure

It was a prospective single centre study. Written informed consent was taken from parents of all children who fulfilled the inclusion criteria. All the patients were thoroughly assessed at presentation, investigated and treated according to the protocol.

Detailed clinical history, demographic profile, anthropometric data and socioeconomic status (Modified Kuppuswamy Classification-2016) was recorded. Address and contact number of patients were also be recorded for further communication. Previous history of tuberculosis, history of contact with pulmonary tuberculosis (within 2 year), vaccination history, past history of medical illness and history of co-morbid illnesses was also taken.

General physical examination and complete systemic examination was done including level of consciousness, signs of meningeal irritation (neck stiffness, kernig's sign, brudzinski's sign), cranial nerve involvement, etc. Chest x-ray, CSF and neuroimaging (CT / MRI brain) was done. Skiagram chest, tuberculin skin test, CBC, ESR, CSF analysis, neuroimaging, CBNAAT of CSF sample and gastric aspirate sample were done in all the patients. A standard tuberculin skin test was performed, and results read after 72 h (positive: induration of >10 mm). All samples were taken with informed consent and under all aseptic conditions and precautions, sent in sterile containers with no time delay.

About 3 ml CSF fluid was drawn by lumbar puncture using standard procedure protocol and 1 ml was sent for CBNAAT test and 2 ml for routine as well as bacteriological examination. CBNAAT was done by using geneXpert machine available in TB clinic of the institute. 1 ml CSF sample is required in clean test vial for CBNAAT testing and sample is to be shifted to lab within 1-2 hr. Transport temperature should be <30ºC. For the test procedure, the sample is poured into a single-use disposable cartridge that is placed in the Xpert Dx module, with the results produced in less than 2 hours. The system automatically interprets all results from measured fluorescent signals, with embedded calculation algorithms, into the following categories: invalid, if PCR inhibitors are detected with amplification failure; negative or positive.

All the information was recorded in predesigned proforma formed in Microsoft excel for final analysis. Bacterial meningitis, viral meningitis and fungal meningitis were ruled out by clinico-radiological, biochemical and bacteriological examinations. CNS tuberculosis was diagnosed based on history, clinical evaluation, CSF finding and neuroimaging findings. Statistical analysis was done using SPSS v20.0 software.

RESULTS

One hundred and ten children between 6 months to 18 years of age with the diagnosis of tubercular meningoencephalitis on the basis of clinical evaluation, CSF examination and neuroimaging were included in the study during the study period of one year (July 2017 to June 2018). Sixty seven children (60.9%) admitted with tubercular meningoencephalitis were in the 1-5 years of age group (60.91%).

CSF was examined in all children with tubercular meningoencephalitis. Majority of children (56.36%) cell counts were in the range of 101-500 cells/µl (>50% lymphocytic pleocytosis presented in all cases). Mean CSF cell count was 198.09±177.86 per µl. 81.82% children CSF protein level was in range of 40-400 mg/dl.

Mean CSF protein level was 230.98±167.73 mg/dl. CSF was analysed for glucose levels. In majority of patients (40%) CSF glucose level was in range of 20-40 mg/dl followed by 31.82% was in range of <20 mg/dl. Mean CSF sugar level was 33.86±18.22 mg/dl. None of the CSF sample and gastric aspirate sample among the study group demonstrated AFB on Ziehl-Neelsen staining.

Table 1: Distribution of cases according Ziehl-Neelsen Staining of CSF and gastric aspirate samples.

Ziehl-Neelsen staining	ZN staining in CSF		ZN staining in gastric aspirate	
	No. of cases	%	No. of cases	%
Negative	110	100%	110	100%
Positive	0	0%	0	0%
Total	110	100%	110	100%

None of the CSF and gastric aspirate sample among the study group demonstrated AFB on Ziehl-Neelsen staining Table 1.

CSF and gastric aspirate were examined by CBNAAT for MTB. 5 (4.55%) children had CBNAAT positivity in CSF. Gastric aspirate was positive among 16 (14.55%) children. None of the patient had CBNAAT positive result both in CSF and gastric aspirate. CBNAAT detected MTB three times more often in gastric aspirate than CSF in diagnosed cases of tubercular meningoencephalitis (Table 2).

Table 2: CBNAAT in CSF and gastric aspirate sample of the study population.

Test result	CBNAAT in CSF		CBNAAT in gastric aspirate	
	No. of cases	%	No. of cases	%
Positive	5	4.55%	16	14.55%
Negative	105	95.45%	94	85.45%
Total	110	100%	110	100%

DISCUSSION

One hundred and ten children between 6 months to 18 years of age with the diagnosis of tubercular meningoencephalitis on the basis of clinical evaluation, CSF examination and neuroimaging were included.

CSF was examined in all children with tubercular meningoencephalitis. None of the CSF sample among the study group demonstrated AFB on Ziehl-Neelsen staining Table 1.

Singh R et al, in their study on clinical profile of pediatric neurotuberculosis.[9] CSF analysis was done in 37 cases out of 46. CSF smear microscopy for acid-fast bacilli were negative in all the cases.

CSF and gastric aspirate were examined by CBNAAT for MTB. Only 5 (4.55%) children had CBNAAT positivity in CSF. Gastric aspirate was positive among 16 (14.55%) children. None of the patient had CBNAAT positive result both in CSF and gastric aspirate. Out of 110 children, this test was positive in 21 (19.1%) patients. Table 2.

Avashia S et al, conducted study on 300 extra pulmonary samples, which included 103 pleural fluids, 81 pus, 45 CSF, 35 Lymph node tissue, 20 ascitic fluids and 16 synovial fluid.[10] out of these 37% (111) patients were Gene Xpert MTB/RIF Assay positive and 36% (40 out of 111) were ZN smear positive. 71 were ZN smear negative but came out to be positive with Gene Xpert assay test, 189 cases were negative for both ZN smear and Gene Xpert MTB/RIF assay. M. tuberculosis was detected in 56.7% (46/81) pus samples, 23.3% (24/103) pleural fluid samples, 54.2% (19/35) lymph node samples, 33.3% (15/45) CSF samples, 20% (4/20) ascitic fluid samples and 18.7% (3/16) synovial fluid samples. The result of the study revealed a maximum positivity rate by Gene Xpert which indicated that it is a more sensitive technique as compared to conventional methods.

Moure R et al, conducted study of 149 smear negative extrapulmonary samples, out of these 108 was culture positive for MTB.[11] Gene xpert detected DNA of MTBC in 63 of the 108 clinical extrapulmonary specimens with MTBC-positive cultures. None of the culture negative sample (41) showed positive result with Gene xpert. Among the 149 specimens studied, 108 specimens had a positive culture of MTBC: (i) 43 liquid specimens (37 sterile fluids, 3 gastric aspirates, and 3 urine specimens) and (ii) 65 nonliquid specimens (34 lymph nodes, 17 abscess aspirates, 12 tissue samples, and 2 stool specimens). Out of 8 gastric aspirate samples 3 were positive for culture, the sensitivity of the Xpert assay was 66.67% (2/3) in culture positive cases and 2/14 CSF samples were culture positive both these culture positive samples were also positive for Gene Xpert assay.

Singh A et al, in 2018 conducted a study to know the role of CBNAAT in diagnosis of tubercular meningitis.[12] 62 patients were included in the study who had features suggestive of tubercular meningitis. According to the universal case definition, the patients were divided into probable, possible and definitive TBM. Out of 62 patients included in the study, 6 (4%) were Definite TBM, 33 (58%) were probable TB, 17 (30%) were possible TBM and 5 (8%) were not TBM. Total 22 patients had M. TB detected in their CSF on CBNAAT, out of a total of 57 TBM patients. The sensitivity of CBNAAT in present study was 38.6%.

Neurotuberculosis is a pauci bacillary disease, number of bacteria are scanty and difficult to demonstrate. Most clinical and neurological manifestation or complication in neurotuberculosis are because of inflammatory immune

response rather than direct damage because of mycobacterium tuberculosis.[13]

CONCLUSION

Gene Xpert assay has the potential to significantly improve and escalate the diagnosis of smear-negative body fluid specimens at both hospitals as well as point-of-care settings in regions with high TB burden.

Negative CBNAAT should not prevent any patient with suspected features of TBME from starting ATT as sensitivity of this test remains low. Final judgement to start ATT should be based on clinical, biochemical and radiological profile especially in CNS tuberculosis.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. WHO. Global Tuberculosis Report. 2018;1-7.
2. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Beyers N. The burden of childhood tuberculosis and the accuracy of community-based surveillance data. Int J Tuberc Lung Dis. 2006;10(3):259-63.
3. Boehme CC, Nabeta P, Hillmann D. Rapid molecular detection of tuberculosis and rifampicin resistance. N Eng J Med. 2010; 363(11):1005-15.
4. Garg RK. Tuberculosis of the central nervous system. Postgrad Med J. 1999;75(881):13-140.
5. Hosoglu S, Geyik MF, Balik. Predictors of outcome in patients with tuberculous meningitis. Int J Tuberc Lung Dis. 2002;6(1):64-70.
6. Lawn SD, Brooks SV, Kranzer K. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. PLoS Med. 2011;8(7):e1001067.
7. Helb D, Jones M, Story E. Rapid detection of Mycobacterium tuberculosis and rifampicin resistance by use of on-demand, near patient technology. J Clin Microbiol. 2010; 48(1):229-37.
8. Rie AV, Shipp PL, Scott L, Sanne I, Stevens W. Xpert MTB/RIF for point of care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope?. Expert Rev Mol Diag. 2010;10(7):937-46.
9. Singh R, Shetty N, Naveed M. Clinical profile of pediatric neurotuberculosis patients at a tertiary care center of Western India. Muller J Med Sci Res. 2018;9(1):12-15.
10. Avashia S, Bansal D, Ahuja K, Agrawal V. Comparison of conventional methods with gene Xpert MTB/RIF assay for rapid detection of mycobacterium tuberculosis and rifampicin resistance in extra-pulmonary samples. Int J Med Res Rev. 2016;4(2):181-5.
11. Moure R, Martín R, Alcaide F. Effectiveness of an integrated real-time PCR method for detection of the *Mycobacterium tuberculosis* complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. J Clin Microbiol. 2012;50(2):513-5.
12. Singh A, Shukla AK, Kaur R. Role of CBNAAT in diagnosis of Tuberculous Meningitis. Int J Curr Res Med Sci. 2018;4(2):59-65.
13. Udani PM. Neurotuberculosis. In: Seth V, Kabra SK, eds. Essentials of Tuberculosis in Children. 4th ed. Jaypee Brothers Publishers; 2011: 150-180.

Cite this article as: Bala S, Goyal S. Study on cartridge based nucleic acid amplification test in children with Neurotuberculosis at RNT Medical College, Udaipur, Rajasthan, India. Int J Contemp Pediatr 2019;6:1588-91.