ORIGINAL ARTICLE
pISSN 0976 3325 | eISSN 2229 6816
Open Access Article
www.njcmindia.org

A STUDY OF POLYMERASE CHAIN REACTION IN CEREBROSPINAL FLUID FOR DIAGNOSIS OF TUBERCULOUS MENINGITIS

Sheena Gupta[1], Alpa Gupta[2], Sanober Wasim[3], Aarti Kotwal[4], Nowneet Kumar Bhat[5]

Financial Support: None declared Swami Rama Himalayan University, Dehradun, Uttarakhand. (Funding done by the university for promoting academic research work)
Conflict of interest: None declared

How to cite this article:
Gupta S, Gupta A, Wasim S, Kotwal A, Bhat NK. A Study of Polymerase Chain Reaction in Cerebrospinal Fluid for Diagnosis of Tuberculous Meningitis. Ntl J Community Med 2016; 7(3):212-215.

Author's Affiliation:
[1]Resident, Dept of Pediatric; [2]Prof, Dept of Pediatric; [3]Asst Prof, Dept of Pediatric; [4]Asso Prof, Dept of Microbiology; [5]Asso Prof, Dept of Pediatric, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun

Correspondence:
Dr. Alpa Gupta
dralpagupta@yahoo.com

Date of Submission: 15-02-16
Date of Acceptance: 01-03-16
Date of Publication: 31-03-16

ABSTRACT

Introduction: Tuberculous Meningitis (TBM) is the commonest type of CNS tuberculosis encountered in children of India. This study was undertaken to evaluate the role of TB Polymerase Chain Reaction (PCR) in CSF for rapid diagnosis of TBM.

Methods: All children having CNS illness and undergoing CSF examination were included in the study and were grouped into two. Group I (Study Group) were subjects with the clinical evidence along with one or more of the laboratory or radiological evidence for tuberculous meningitis. Group II (Control Group) comprised of subjects who were diagnosed to have CNS illness other than tuberculous meningitis undergoing CSF examination. CSF samples from all the subjects were subjected to AFB staining, culture and PCR for Mycobacterium tuberculosis.

Results: A total of thirty three subjects were enrolled in the study of which 18 were in Group I and 15 were in Group II. The sensitivity and specificity of TB PCR for diagnosis of TBM was 77.78% & 100%, respectively and positive predictive value was 100% and the negative predictive value was 78.95%.

Conclusion: Analysis of TB PCR in CSF is a sensitive tool with high specificity for making rapid and accurate diagnosis of TBM.

Key words: Polymerase chain reaction, tuberculous meningitis, TBM diagnosis, Mycobacterium tuberculosis

INTRODUCTION

Tuberculosis (caused by Mycobacterium tuberculosis) is one of the most widespread infections affecting almost 1/3rd of world's population. It is an important cause of mortality and morbidity among both adults and children especially in developing countries[1]. Central Nervous system (CNS) tuberculosis is the severe form of *Mycobacterium tuberculosis* infection, causing death or severe neurological defects in more than half of those affected[2]. Tuberculous Meningitis (TBM) is the commonest type of CNS tuberculosis encountered in children of India[3]. Incidence of TBM varies from 1-4 % of total inpatient admissions in different parts of India[3]. About 10% of patients who have tuberculosis develop CNS disease[3].

Owing to its relative wide spectrum of neurological symptoms, CNS tuberculosis remains a formidable challenge. Accurate, rapid diagnosis and early treatment are the most important factors with

regard to the prognosis and the prevention of long-term neurological sequelae in tuberculous meningitis[2].

Various tests are available to clinician to aid in making diagnosis of TBM. Mantoux test, radiological tests (X-Ray, CT scan, MRI) and CSF studies (sugar, protein and cytological analysis) have supportive role. Definitive diagnosis is made by demonstration of mycobacteria in CSF by direct AFB staining or culture. CSF culture for mycobacterium is time consuming. The low sensitivity of AFB staining in CSF has been demonstrated in various studies, Thwaites et al had reported it to be 52%[5] and Zhuang et al reported its positivity rate as 8.6% [7]. For the CSF culture Cruciani et al in a meta-analysis reported the sensitivity for detecting mycobacteria to be 81.5%[13].

The Polymerase Chain Reaction (PCR) is a versatile technique and is increasingly being used in rapid diagnosis of TB. This study was undertaken to evaluate the role of TB PCR in CSF in the diagnosis of tuberculous meningitis.

METHOD

Our study is a hospital based observational analytical study conducted in the Department of Pediatrics, Himalayan Institute of Medical sciences (HIMS), Swami Rama Himalayan University (SRHU), Dehradun, over a period of twelve months from January'2014 to December'2014 with the aim to determine sensitivity and specificity of Polymerase chain reaction in CSF of children suffering from TBM. All children having CNS illness who were undergoing CSF examination were included in the study. The children where consent for CSF examination was not given by the attendants and children with congenital CNS malformations were excluded.

All CSF samples were sent for routine biochemical and cytological analysis, PCR for TB, AFB staining and culture for tubercular bacilli. Mycobacterium culture was done using Mycobacterium Growth Indicator Tube **(**MGIT) in BACTEC 460 (Becton Dickinson, Sparks, MD, USA). TB PCR was done using AccuPower MTB & NTM Real – Time PCR Kit.

The subjects were divided into two groups - Group I (Study Group) and Group II (Control Group). Group I (Study Group) comprised of the subjects with the clinical evidence along with one or more of the laboratory or radiological evidence for tuberculous meningitis. Clinical evidence for TBM was fever with or without headache for more than 2 weeks duration.Laboratory evidence for TBM were **(i)** CSF study suggestive of TBM : WBC > $20/mm^3$ (with lymphocytic predominance), proteins more than 100mg/dl, sugar < 45 mg/dl or <40% of blood glucose level; **(ii)** radiological evidences (CECT/ MRI Brain) like basal exudates, hydrocephalus, tuberculoma, focal brain abnormalities such as infarction; **(iii)** associated TB outside the CNS or positive PPD skin test. Group II (Control Group) comprised of subjects who were diagnosed to have CNS illness other than tuberculous meningitis undergoing CSF examination and not fulfilling the criteria for Group I.

Statistical Methods: Statistical analysis was performed with SPSS Version 20. Fisher's exact test was done to measure degree of association between categorical variables. Statistical significance was set at $P < 0.05$.

RESULTS

A total of thirty three subjects were enrolled in the study of which 18 were in Group I (Study group) and 15 were in Group II (Control group). The demographic characteristics of both the groups are given in Table 1. The difference in the mean age in the two groups and the male:female ratio was found to be statistically not significant. In Group I 83.33% of the subjects had malnutrition. In Group II 40% of subjects were malnourished. This difference in the two groups was statistically significant (p value= 0.01).

The difference in history of contact (with a patient with tuberculosis) amongst the two groups was statistically significant with more subjects in Group I having a positive history of contact with tuberculosis patient.

Table 1: Demographic characteristics of Group I and Group II

	Group I (n=18)	Group II (n=15)	P value
Age in years* (SD#)	11.58 (4.82)	10 (5.34)	0.19
Gender (Males : Females)	11 : 7	6 : 9	0.302
Place of residence (Rural : Urban)	13: 5	7 : 8	0.168
Positive History Of Contact with patient with tuberculosis	9 (50%)	1 (6.67%)	0.009
BCG$ Vaccination Received	11 (61.11%)	11 (73.33%)	0.71
Malnutrition present	15 (83.33%)	6 (40%)	0.01

*mean age ; #: Standard Deviation; $: Bacilllus Calmette Guerin; Group I: Study Group; Group II: Control Group

Table 2: Clinical and laboratory data of children in Group I

Clinical & Lab Data	**Children (n=18)(%)**
Meningeal Signs	12 (66.67)
Cranial Nerve Palsies	8 (44.44)
Reactive Mantoux	9 (50.00)
Positive Radiological Finding	13 (72.22)
CSF Studies suggestive of tuberculous meningitis	18 (100)
Positive TB PCR in CSF	14 (77.78)
CSF Smear Positive For AFB	7 (38.89)
CSF culture positive for M.tb	13 (72.22)

CSF: Cerebrospinal fluid; TB: Tuberculosis; PCR: Polymerase chain reaction; AFB: Acid Fast Bacilli; M.tb: Mycobacterium tuberculosis.

In Group I 11 (61.11%) subjects had received BCG vaccination while 7 (38.89%) subjects were not immunized. In Group II 11 (73.33%) subjects had received BCG immunization. There was no statistically significant difference in BCG vaccination status in the two groups (p value= 0.71).

CSF examination was suggestive of tuberculous meningitis in all of the subjects in Group I. Acid Fast tubercular Bacilli (AFB) were seen in the CSF smear of 7 (38.89%) subjects in Group I. None of the subjects in Group II had CSF smear positive for AFB. For the diagnosis of tuberculous meningitis the sensitivity and specificity of AFB staining in CSF was found to be 38.89% and 100% respectively. Its Positive predictive value was observed to be 100% and Negative predictive value was observed to be 57.69%. In Group I CSF culture was positive for Mycobacterium tuberculosis in 13 (72.22%) subjects and it was negative in 5 (27.78%). None of the subjects in Group II had CSF culture positive for Mycobacterium tuberculosis. The sensitivity and specificity of Mycobacterium tuberculosis culture in diagnosis of tuberculous meningitis, was found to be 72.22% and 100% respectively. Its Positive predictive value was observed to be 100% and Negative predictive value was observed to be 75%.

In Group I TB PCR was positive in CSF of 14 (77.78%) subjects while in Group II none of the subjects had positive TB PCR in CSF. The sensitivity and specificity of TB PCR in CSF for diagnosis of tuberculous meningitis was found to be 77.78% and 100% respectively. The Positive predictive value was observed to be 100% and the Negative predictive value was observed to be 78.95%.

DISCUSSION

In tuberculous meningitis accurate, rapid diagnosis and early treatment are the most important factors with regard to the prognosis and prevention of long-term neurological sequelae[2]. Delay in diagnosis and so in the start of effective treatment results in poor prognosis and sequelae in up to 25% of cases. Conventionally, TBM is diagnosed by cytological and biochemical analysis of CSF. Definite diagnosis is made by demonstration of mycobacteria in CSF by AFB staining or culture[4]. Evidences like positive Mantoux test and radiological findings play a supportive role. Partially treated pyogenic meningitis is a formidable challenge in making correct and rapid diagnosis especially if supportive evidences are not present as CSF is seldom positve for AFB (sensitivity is 52%) and cultures take a long time[5].

Recently, the detection of *Mycobacterium tuberculosis* DNA in the CSF through the use of various molecular-based methods, including nucleic acid amplification (NAA) assay technique, particularly polymerase chain reaction (PCR) assay, has emerged as a promising new method for the diagnosis of CNS tuberculosis because of its rapidity and high specificity. Many investigators have reported the usefulness of PCR assay for the detection of *Mycobacterium tuberculosis* DNA in CSF[2].

The polymerase chain reaction (PCR) is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies. Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications[6].

In our study sensitivity and specificity of AFB staining in CSF was found to be 38.89% and 100% respectively. Positive predictive value was 100% and Negative predictive value was 57.69%. Our findings were in accordance to the study by Thwaites et al who reported that the sensitivity, specificity of AFB staining in CSF were 52%, 100%, respectively[5]. The low sensitivity of AFB staining in CSF has been demonstrated in various studies. The Study of Zhuang et al showed that the positivity rate for detection of AFB in CSF in patients with clinical diagnosis of TBM was 8.6% [7]. In a study Kox et al reported the sensitivity of microscopy to be 9%[6]. In their study, Chacko et al stated that AFB staining in CSF is not sensitive enough to help the clinician in ruling out the possibility of TBM[9]. Bhigjee et al reported that the CSF of most patients with tuberculous meningitis contains only 10^0-10^2 organisms/ml. Approximately 10^4 organisms/ml are required for reliable detection with AFB staining. The detection rate in CSF smears can be improved by taking large volumes of CSF and spinning it at high speeds for a prolonged period[10]. Katti et al have recommended that collection of four serial samples and spinning of large volumes (10–20 ml) of CSF for 30 minutes could enhance the

rate of detection in smear microscopy. However, repeated collection of such large volume of CSF is practically not possible[11]. Hence demonstration of AFB in CSF by microscopic examination is an unreliable tool for diagnosis of TBM.

In our study the sensitivity and specificity of Mycobacterium tuberculosis culture in diagnosis of tuberculous meningitis was found to be 72.22% and 100% respectively. The Positive predictive value and negative predictive value was found to be 100% and 75% respectively. Thwaites et al also reported a sensitivity of 71%[12]. In a meta analysis, Cruciani et al found that the sensitivity and specificity of culture for detecting mycobacteria is 81.5% and 99.6% respectively[13]. Kumar et al concluded that bacteriological proof of tuberculous meningitis is available in only a small proportion of patients because the yield of CSF culture for mycobacteria is generally low[14].

In the literature, studies evaluating the role of PCR for detection of Mycobacterium tuberculosis have demonstrated variable sensitivity (30–90%) and specificity (95–100%) [15]. In our study in Group I TB PCR was positive in CSF of 77.78% of subjects while in Group II none of the subjects had positive TB PCR. Bhigjee et al reported that the sensitivity of TB PCR in diagnosis of tuberculous meningitis was 70.5% whilst the specificity was 87.5%[10]. Study published by Kulkarni et al reported the overall sensitivity and specificity of the TB PCR assay in CSF was respectively 90% and 100%; the positive predictive value was 100% and the negative predictive value was 90 % [16]. The sensitivity and specificity of PCR in our study was 77.78% and 100% respectively with positive and negative predictive values of 100% and 78.95%, respectively.

CONCLUSION

The rapid and accurate diagnosis of TBM with conventional CSF analysis remains a challenge. PCR having a high specificity of 100% and positive predictive value of 100% can be used as a valuable and a powerful aid in this regard especially in cases with clinical dilemma like partially treated pyogenic meningitis. A combination of clinical criteria and PCR can enhance the early diagnosis and hence improve the management and outcome of TBM.

Acknowledgements : Mr Hem Sati, (Department of Community Medicine, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand) for helping in analysis of the data.

REFERENCES

1. Seth V, Kabra SK. Essentials of tuberculosis in children. 2011;4.
2. Kaneko K, Ondera O, Miyatake T, Tsuji S. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. Neurology. 1990; 40: 1617-8.
3. Murthy J. Tuberculous meningitis: The challenges. Neurol India 2010;58:716-22
4. Daniel TM. New approaches to the rapid diagnosis of tuberculous meningitis. J Infect Dis 1987; 155: 599–603.
5. Thwaites GE, Caws M, Chau TTH, Dung NT, Campbell JI, Phu NH, Hien TT et al. Comparison of conventional bacteriology with nucleic acid amplification (amplified mycobacterium direct test) for diagnosis of tuberculous meningitis before and after inception of antituberculosis chemotherapy. J Clin Microbiol 2004; 42:996-1002.
6. Bartlett JMS, Stirling D. A Short History of the Polymerase Chain Reaction. PCR Protocols. 2003; 226: 3–6.
7. Zhuang YH, Lin GL, Zhang XG. Evaluation of detection of M. tuberculosis by DNA amplification. Zhonghua Jie He He Hu Xi Za Zhi. 1993; 16: 26-9.
8. Kox LF, Kuijper S, Kolk AH. Microwell hybridization assay for detection of PCR products from Mycobacterium tuberculosis complex and the recombinant Mycobacterium smegmatis strain 1008 used as an internal control. J Clin Microbiol. 1996; 34(9): 2117–2120.
9. Chacko F, Modi M, Lal V, Prabhakar S, Rana SV, Arora SK. Diagnostic efficacy of adenosine deaminase levels in cerebrospinal fluid in patients of tubercular meningitis: A comparison with PCR for Mycobacterium Tuberculosis. Annals of Neurosciences 2010; 17(3):
10. Bhigjee AI, Padayachee R, Paruk H, Devi K, Pillay H, Marais S, et al. Diagnosis of tuberculous meningitis: clinical and laboratory parameters. International Journal of Infectious Diseases. 2007; 11: 348—54.
11. Katti MK. Pathogenesis, diagnosis, treatment, and outcome aspects of cerebral tuberculosis. Med Sci Monit. 2004; 10: 215–29.
12. Thwaites GW, Chau TTH, Stepniewska K. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. The Lancet 2002; 360(9342):. 1287–1292.
13. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycbacteria. J Clin Microbiol 2004; 42:2321–25.
14. Kumar R, Singh SN, Kohli N. A diagnostic rule for tuberculous meningitis. Arch Dis Child. 1999; 81:221–4.
15. Mahadevan B, Mahadevan S, Serane VT, Narasimhan R. Tuberculin reactivity in tuberculous meningitis. Indian J Pediatr. 2005 Mar;72(3):213-5.
16. S. P. Kulkarni, M. A. Jaleel, G. V. Kadival. Evaluation of an in-house-developed PCR for the diagnosis of tuberculous meningitis in Indian children. J. Medical Microbiol. 2005; 54(4): 369–373.