ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.30636-30639, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

UTILITY OF REAL TIME PCR IN CULTURE NEGATIVE ACUTE BACTERIAL MENINGITIS IN PEDIATRIC AGE GROUP

***,[1]Prakash Kumar Mishra, [2]Kaur, I. R., [2]Manchanda, V., [3]Batra, P. and Singh, N. P.**

[1]Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden
[2]Department of Microbiology, Chacha Nehru Bal Chikitsalaya, Geeta Colony, Delhi
[3]Department of Pediatrics, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden

ARTICLE INFO

Article History:
Received 25th February, 2016
Received in revised form
14th March, 2016
Accepted 15th April, 2016
Published online 10th May, 2016

Key words:
Real Time PCR,
Culture negative Acute Bacterial Meningitis,
Pediatric Age Group.

ABSTRACT

Introduction & objective: Acute Bacterial Meningitis (ABM) is a medical emergency which warrants early diagnosis. Pretreatment with antibiotic before lumbar puncture make interpretation of CSF culture difficult. Clinicians must rely on the other tests to determine the etiology of meningitis. Thus it is very important to ascertain the diagnostic efficacy of different tests employed. This study was aimed to evaluate Real Time PCR with other diagnostic methods in culture negative ABM in pediatric age group.

Material & Method: 60 CSF samples from suspected cases of ABM were subjected to cytology, gram staining, biochemical analysis, culture as well as antigen detection by latex agglutination test (LAT) for *S.pneumoniae*, *H.influenzae*, *N.meningitidis*, *E.coli*, *Gr.B Streptococcus* and probe based Real Time PCR for *S.pneumoniae*, *H.influenzae*, *N.meningitidis*.

Result: Among the five PCR positive samples for *S. pneumoniae* four were positive by LAT, of which only one was isolated by culture. Of the three PCR positive *H.influenzae* samples, two were positive by LAT but none yielded growth. All CSF samples demonstrating microorganism by any means (culture, LAT, RTPCR) had shown Polymorphonuclear leucocytosis & pus cells.

Conclusion: This study emphasizes the need to subject pediatric CSF samples to Real Time PCR for identifying microorganism, showing polymorphonuclear leucocytosis & pus cells which may be missed on culture.

Citation: Prakash Kumar Mishra, Kaur, I.R., Manchanda, V., Batra, P. and Singh, N.P., 2016. "Utility of real time pcr in culture negative acute bacterial meningitis in pediatric age group", *International Journal of Current Research*, 8, (05), 30636-30639.

INTRODUCTION

Acute Bacterial Meningitis (ABM) is one of the most severe infectious diseases in pediatric age group. There is a need for periodic review of ABM, since the pathogens causing the infection vary with time, geography and patient age (Tang *et al*., 1999). Choice of initial antibiotic therapy is based on this. Pretreatment with antibiotic before lumbar puncture hampers interpretation of CSF culture (Riordan, 2002). Clinicians must rely on the other tests to determine the etiology of meningitis. Thus it is very important to ascertain the diagnostic efficacy of different tests employed.

****Corresponding author: Prakash Kumar Mishra***
Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden

Conventional methods take 48-72 hours for isolation and identification of the causative bacteria of ABM. Delay in diagnosis and initiation of antimicrobial therapy can result in poor outcome of the disease. Microscopy, antigen detection and nucleic acid amplification techniques may help in early diagnosis of ABM. This study was aimed to evaluate Taqman probe based Real Time PCR (RTPCR) with other diagnostic methods in case of culture negative ABM in pediatric age group.

MATERIALS AND METHODS

An observational study was done at Departments of Microbiology & Pediatrics, U.C.M.S. & G.T.B. Hospital, Delhi after approval from Institutional ethical committee. CSF from 60 provisionally diagnosed cases of ABM in Pediatric Casualty and NICU were taken. CSF samples were macroscopically

examined & direct cytology was done. CSF sample was centrifuged and smear was prepared from deposit for demonstration of pus cells and microorganism on Gram staining. Detection of soluble antigen of *H.influenzae* type b, *Streptococcus pneumoniae*, *Neisseria meningitidis* group A, groupB/*E.coli* K1, group C, group Y/W135 & *Streptococcus* group B in CSF sample was performed by latex agglutination test (LAT) using PASTOREX ™ Meningitis kit (BIO-RAD). CSF sample were cultured & isolates were identified as per standard protocol (Collee *et al*., 1996). All the isolates were tested for antimicrobial susceptibility as per CLSI guideline (Cockerill *et al*., 2010) From each CSF sample DNA was extracted by Accuprep® Genomic DNA Extraction Kit (BIONEER) and subjected to TaqMan probe based Real time PCR for detection of *S. pneumoniae* ,*H.influenzae* and *N.meningitidis* (Corless *et al*., 2001). (Table1 & Table 2). Levels of protein & glucose in CSF were estimated by automated analyzer as per manufacture instruction (Star21 plus, Rapid Diagnostics Pvt ltd, Mumbai).

26(43.33%) showed pus cells and all these samples showed polymorphonuclear leucocytosis but only 16 showed turbid appearances. In one sample only lanceolate shaped Gram positive cocci in pairs could be seen. By antigen detection assay (LAT), 4 each were positive for *Streptococcus pneumoniae* and *Group B Streptococcus,* 2 were positive for *Haemophilus influenzae* and 1 was positive for *Escherichia coli*. In LAT positive 11 samples only 6(54.54%) were turbid whereas all had shown polymorphonuclear leucocytosis and pus cells.LAT were positive in 9 culture negative CSF samples. CSF protein was more than normal level in 25 (41.66%) samples. CSF glucose level was low in 20 (33.33%) samples. In 5(8.33%) of the 60 CSF samples, microorganism were isolated. 2 were identified as *Acinetobacter baumanii,*1 each as *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella oxytoca*. All culture positive CSF samples were turbid and had shown polymorphonuclear leucocytosis and pus cells.

Table 1. Bacteria & Primer, probe sequences were as follows

Bacteria	Primer sequence
Neisseria meningitidis	CtrA F- 5´-gCTgCggTAggTggTTCAA
	CtrA R- 5´-TTgTCgCggATTTgCAACTA
	CtrA TM- 6FAM-CATTgCCACgTgTCAgCTgCACAT-BBQ
Hemophilus influenzae	gyrA.S F- 5´-CACTTCgCTATATgTTggTTgAT
	gyrA.A R- 5´-CCATCATAgTTTggCgAgAA
	Hinflu.TM- 5´-6 FAM- CTgCAATgCgTTATACCgAAgTgc-BBQ
Streptococcus pneumoniae	ply F- 5´-TgCAgAgCgTCCTTTggTCTAT
	ply R- 5´-CTCTTACTCgTggTTTCCAACTTgA
	ply TM- 5´-6 FAM-TggCgCCCATAAgCAACACTCCgAA--BBQ

Table 2. PCR Parameters for molecular detection of *N.miningitidis,S.pneumoniae,H.influenzae*

Programme name	cycle	Temp(^{o}c)	Holding time	Ramp rate(^{0}C/Sec)
Preincubation-denaturation	1	95	5 min	4.4
Amplification	50*/45$^{\#}$/50$^{\$}$	Denaturation-95	10 sec	4.4
		Annealing-60*/59$^{\#}$/54$^{\$}$	40*/20$^{\#}$/40$^{\$}$ sec	2.2
		Extension-72	01*/20$^{\#}$/01$^{\$}$	4.4
Cooling	1	40	10 sec	2.2

**N.menigitidis, #S.pneumoniae, $H.influenzae*

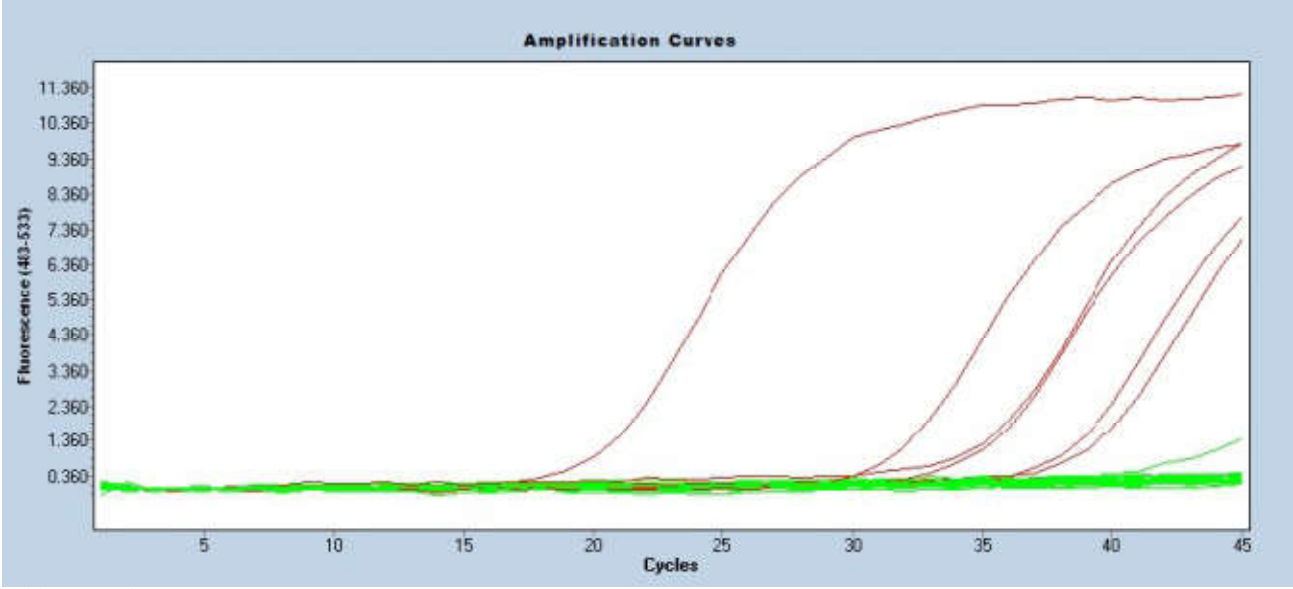
Table 3. Evaluation of Real Time PCR for *Streptococcus pneumoniae* and *H.influenzae* in comparison to LAT & culture

Real Time PCR (n)	*S. pneumoniae*				*H. influenzae*			
60	RT PCR Positive	Culture positive LAT positive	Culture negative LAT positive	Culture negative LAT negative	RT PCR Positive	Culture positive LAT positive	Culture negative LAT positive	Culture negative LAT negative
	5	1	3	1	3	0	2	1
	Negative 55		55		Negative 57		57	

RESULTS

Out of the 60 pediatric cases provisionally diagnosed as ABM, 39 (65%) were males and 21 (35%) were females.17(28.3%) were neonate,17 (28.3%)were 29 days to 1 year of age,12(20%) were >1 year to 5 years of age and 14 (23.4%)were >5 years to 12 years of age. All were empirically treated with antibiotic before lumbar puncture was done. 16(26.66%) CSF samples were turbid and 27(45%) had shown polymorphonuclear leucocytosis on cytological examinations. On Gram staining

By Real Time PCR (RTPCR), 8 out of the 60 sample (13.3%) could be identified as *Streptococcus pneumoniae* (5/60) & *Haemophilus influenzae* (3/60). (Table 3) (Figure1) RTPCR were positive in 2 LAT negative CSF samples one each for *S.pneumoniae* & *H.influenzae.* RTPCR were positive in 7 culture negative CSF samples, 4 for *S.pneumoniae* and 3 for *H.influenzae*. There was no any sample in which RTPCR was negative and culture was positive. Both RTPCR positive and LAT negative CSF samples were clear and positive for polymorphonuclear leucocytosis and pus cells.



PC-Positive Control

Figure 1. Real Time PCR Curves for *S. pneumoniae*

DISCUSSION

ABM is a medical emergency, which warrants early diagnosis and aggressive therapy. In this study males were more commonly affected than females as in other studies. The impact of sex hormones on the T-helper 1/T-helper 2 cytokine balance has been proposed to explain the higher gender predilection towards male. (Muenchhoff and Goulder, 2014; Gaegas *et al*., 1977-1978; Saleh *et al*., 1991) This study showed neonates were most commonly affected. High incidence rate of ABM among this age group is due to the immature immune system of neonates, colonization of the organisms in the female genital tract and the increase of the permeability of the blood brain barrier. Several studies had reported the similar findings. (Ahmed *et al*., 1996; Almoneef *et al*., 1998) Out of 60 CSF samples collected 16(26.66%) samples were turbid on macroscopic examination and from these samples microbes were isolated in only 5 (8.33%) cases by conventional culture. 46% LAT positive & 50% PCR positive samples were clear, which indicates that turbid CSF cannot be taken as a sole marker for ABM as also observed by Garges *et al*. (2006) Cytology & Gram staining of CSF can offer immediate clues to aid a diagnosis of ABM. Some studies have reported a CSF Gram stain sensitivity of 60-90% and a high specificity of >97%, stressing its importance in the rapid and accurate diagnosis of the causative bacteria. (Neuman *et al*., 2008) The yield of bacteria on a Gram stain depends on several factors like the number of organisms present, prior use of antibiotics, technique used for smear preparation, staining techniques and the observer's skill and experience. In this study, all CSF samples demonstrating microorganism by any means (culture, LAT, RTPCR) has shown Polymorphonuclear leucocytosis & pus cells. This study advocates cytology & Gram staining of CSF as a routine procedure for detection of pus cells as an indicator of bacterial infection.

CSF protein was elevated in 25 (41.66%) samples and CSF glucose level was low in 20(33.33%). All cases which were culture, LAT, Real Time PCR positive, had elevated CSF protein & low CSF glucose levels. It has been observed in many studies that ABM can occur in presence of normal CSF protein and glucose levels. (Garges *et al*., 2006) CSF samples from 5 (8.33%) patients were positive on culture in this study. Several studies report culture negative cases of meningitis or a low CSF culture positivity, ranging from 6 to 50%. (Bhat *et al*., 1991; Das *et al*., 2003) Various reasons cited in the literature for a low yield of bacteria on culture are prior antibiotic therapy, delay in transport of specimens to the laboratory, non availability of special media for specific pathogens, presence of autolysis enzymes in CSF and lack of a 24 hour facility for processing CSF samples. (Sonavane *et al*., 2008) Antibiotic administration prior to LP was done in all the suspected cases of ABM in this study which could be one of the main reason for low yield of microorganism. Though the common pathogens associated with ABM are *S. pneumoniae, H. influenzae and N. meningitidis*, the etiological agents and their relative frequency may vary in different geographical areas. As compared to Western studies, the relative incidence of meningitis caused by *H.influenzae, N. meningitidis* and *Listeria* is less in South-East Asia. On the contrary, gram negative bacilli are increasingly being recognized as important pathogens of ABM, as also observed in this study. (Kumar *et al*., 2007) Group B *Streptococcus* antigen could be detected in the CSF sample of 4 neonates by LAT. Group B streptococci, one of the most important causes of ABM in western countries is being increasingly recognized as a cause of neonatal meningitis in Southeast Asia. (Wilder-Smith *et al*., 2000; Berman *et al*., 2004) By Real Time PCR seven additional cases, which were not diagnosed by the conventional culture techniques could be detected. RTPCR additionally detected microorganism in two LAT negative CSF samples. Probe based assay are more specific than SYBR Green. (Malinen *et al*., 2003) The disadvantage with SYBR Green is that, it will bind to any double-stranded DNA in the reaction, including primer-dimers and other non-specific reaction products, which results in an overestimation of the target concentration. Probe based Real time Polymerase chain reaction provides fast, precise and accurate results. Different studies have showed a high

specificity of the assay. (John *et al*., 2001) The present study emphasizes the need to subject pediatric CSF samples to Taqman probe based Real Time PCR for identifying microorganisms, especially in CSF samples showing polymorphonuclear leucocytosis & pus cells which may be missed on culture.

Acknowledgement

We thank the study participants, parents and guardians of study participants and study staff for their support and participation in the study.

REFERENCES

Ahmed AA, Saleh MA, and Ahmed HS. Post-endemic acute bacterial meningitis in Sudanes children, *East Afr Med*., J1996; 73: 527-532.

Almoneef MJ, Memish Z, KhonY, Kagall WA and Shaalan M. Childhood meningitis in Saudi Arabia, *J Infect*, 1998*;* 2: 157-160.

Berman RE, Kliegman RM, Arvin AM. Nelson Textbook of Pediatrics.17th ed. Philadelphia, PA: WB Saunders; 2004

Bhat BV, Verma IC, Puri RK, Srinivasan S, Nalini P.A profile of pyogenic meningitis in children. *J Indian Med Assoc*., 1991; 89(8):224-7.

Cockerill FR, Wikler MA, Bush K, Dudley MN, Eliopoulos GM, Hardy DJ, *et al*. Performance standards for antimicrobial susceptibility testing. *CLSI*, 2010;30(1):40-104.

Collee JG, Marr W, Fraser AG, Marmion BP, Simmons A. Mackie & McCartney Practical Medical Microbiology, 14thed.Delhi:Churchill Livingstone;1996.

Corless CE, Guiver M, Borrow R, Jones VE, Fox AJ, Kaczmarski EB. Simultaneous detection of *Neisseria meningitidis, Haemophilus influenzae* & *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time pcr. *J Clin Microbiol*., 2001 Apr; 39(4):1553-58.

Das BK, Gurubacharya RL, Mohapatra TM, Mishra OP. Bacterial antigen detection test in meningitis. *Indian J Pediatr*., 2003;70 :799-801.

Gaegas N, Hafed K, Aziz Al Khawlly M, Robenz GB, Gotshelish A. Bacterial meningitis in Egypt: Serotyping of the isolated bacteria from CSF samples of the patients in tow hospitals of Cairo, 1977-1978. Bulletin of the WHO; 61: 501-512.

Garges HP, Moody MA, Cotton CM, Smith PB, Tiffany KF, Lenfestey R. *et al*. Neonatal Meningitis: What Is the Correlation Among Cerebrospinal Fluid Cultures, Blood Cultures, and Cerebrospinal Fluid Parameters? Pediatrics 2006; 117: 1094-1100.

John AJP, Lalitha MK, Cherian T, Pai R, Thomas K, Steinhoff MC. A polymerase chain reaction- enzyme immunoassay for diagnosis of pneumococcal meningitis in children and adults. *Indian J Med Res*., 2001; 113:48-52.

Kanegaye JT, Soliemanzadeh P and Bradley JS. Lumbar puncture in pediatric bacterial meningitis: determining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics*, 2001; 108: 1169-74.

Kumar S, Kashyap B, Bhalla P . The rise and fall of epidemic Neisseria meningitidis from a tertiary care hospital in Delhi, January 2005-June 2007. *Trop Doctnone.*, 2008; 38:222-4.

Malinen E, Kassinen A, Rinttila T, Palva A.Comparison of real-time PCR with SYBR Green I or 5′-nuclease assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in quantification of selected faecal bacteria. *Microbiology*, (2003); 149:269–277.

Muenchhoff M, Goulder PJR. Sex Differences in Pediatric Infectious Diseases. *JID*, 2014;209 (Suppl 3) :120-126.

Neuman MI, Tolford S, Harper MB. Test characteristics and interpretation of cerebrospinal fluid gram stain in children. *Pediatr Infect Dis J*., 2008;27(4):309-13.

Riordan FAI, Cant AJ. When to do a lumbar puncture. *Arch Dis Child*., 2002; 87: 235-7.

Saleh MA, Khaleefa OH, Mohmed B, Zubair BT, Musa ZA, Kamil Izzedin *et al*. Long-term sequelae of childhood acute bacterial meningitis in developing country, *Scand J Infect Dis*., 1991 23: 175-182.

Sonavane A, Baradkar VP, Mathur M. Bacteriological Profile of Pyogenic Meningitis in Adults. *Bombay Hospital Journal*, 2008;50 (3):452-5.

Tang LM, Chen ST, Hsu WC, Lyu RK. Acute bacterial meningitis in adults: A hospital- based epidemiological study. *QJM,* 1999; 92: 719-25.

Wilder-Smith E, Chow KM, Kay R, Ip M, Tee N. Group B Streptococcal meningitis in adults: Recent increase in Southeast Asia. *Aust N Z J Med*., 2000; 30: 462-5.

****