Original Article

Comparative Study of Polymerase Chain Reaction (PCR) and Conventional Methods for the Diagnosis of Pneumococcal Meningitis in CSF of Under Five Children in Chattogram Medical College, Chattogram.

***Rozario DTD[1]**

Abstract

Background: *Bacterial meningitis is an important cause of mortality and long term morbidity. Early and accurate diagnosis of bacterial meningitis is of critical concern. Though bacterial culture is considered as gold standard, this approach has some disadvantages with regard to rapidity and sensitivity. This has motivated the evaluation of alternative diagnostic strategy.* ***Objectives:*** *This study was performed to compare between polymerase chain reaction (PCR) and conventional methods for the diagnosis of pneumococcal meningitis in under five children.* ***Materials & Methods:*** *This cross sectional study was carried out in the Department of Microbiology, Chittagong Medical College for cytological examination, biochemical tests, Gram's stain, culture, and PCR for lytA gene of Streptococcus. pneumoniae in CSF.* ***Results:*** *Among the 68 cases of probable bacterial meningitis, culture was positive in 22 (32%) and Gram's stain was positive in 17 (25%) cases. Streptococcus pneumoniae was the predominant organism detected by isolation in 11 (50%). PCR detected 27 (46.67%) cases of S. pneumoniae among 57 bacterial meningitis cases. All the culture and Gram's stain positive cases for Streptococcus pneumoniae were also positive by PCR. The Sensitivity, Specificity, Positive predictive value and Negative predictive value of PCR were 100%, 65%, 41%, and 100% respectively by using CSF culture as gold standard.* ***Conclusion:*** *PCR was highly sensitive and specific and PCR was found superior to other available methods for detection of bacterial meningitis.*

Keywords: CSF, PCR, Pneumococcal meningitis

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Introduction

Acute bacterial meningitis (ABM) is one of the most dramatic medical emergencies which is seen as a public health challenge worldwide. The disease is dreaded for its acute devastating onset in previously healthy individuals and difficulty in obtaining a timely and accurate diagnosis.[1] Globally 1.2 million cases of bacterial meningitis are estimated to occur every year with 1,35000 deaths.[2] The disease is much more common in developing countries than the developed countries. Gurley et al. (2009) from Bangladesh reported that among all meningitis cases bacterial meningitis constitutes 25% and case fatality rate was 14%.[3] The bacterial meningitis epidemiological landscape is not static and etiological agent varies with age and immune status and different geographic area. Incidence of confirmed Hib meningitis in Bangladeshi infants was 92/100000 in pre vaccine period. The incidence dramatically declined to 15.7 cases/100000 children a year after introduction of the vaccine.[4] So except during an epidemic of meningococcal infection, Streptococcus pneumoniae is the commonest cause of acute bacterial meningitis.[5] Because of the high mortality and morbidity resulting from bacterial meningitis, rapid and accurate diagnosis is needed to increase the survival rate and decrease complications. Though Gram's stain is simple, rapid and less expensive method for detecting bacteria but it has some limitations. The yield of bacteria on a Gram's stain depends on several factors like the number of organisms present, prior use of antibiotic, technique used for smear preparation (centrifuged deposit, cytospin, direct smear etc.). The gold standard for diagnosis of any infection including meningitis is the isolation and identification of the causative agent.[6] But it requires a day or more for growth and can also give false negative result

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due to the preceding antibiotic therapy before admission or meningitis due to fastidious organisms.[1] The increasing practice of preadmission administration of parenteral antibiotic therapy and reluctance to perform lumber puncture at admission are pointed out to contribute a decrease in culture confirmed cases in several countries.[7] So an alternative method for the diagnosis of bacterial meningitis is required which is rapid, reliable, less time consuming, easy to perform, sensitive and specific. Polymerase chain reaction (PCR) is highly sensitive and specific technique for diagnosis of bacterial meningitis.[8] PCR now can detect low number of pathogens in clinical specimens which does not require the presence of viable organisms.[9] So the purpose of the study was to determine the frequency of pneumococcal meningitis in under five children, to assess the diagnostic efficacy of PCR in identifying *lytA* gene of Streptococcus.

Materials and Methods

A total of 272 clinically suspected patients of meningitis of age ranging from 0 day to 5 years from Neonatal, Pediatrics wards and Medicine wards of CMCH and CMOSH, Chittagong were included in this study. This cross sectional descriptive study was carried out during the period of July 2019 to June 2020. Ethical clearance was duly obtained from Ethical Review Committee, Chittagong Medical College, Chittagong.

Clinically suspected patients of meningitis with high body temperature, signs of meningeal irritation, i.e. neck rigidity, Kernig's sign, Brudzinski's sign, headache, vomiting, altered level of sensorium, high pitched crying and photophobia were included in this study. Patients treated with injectable antibiotics for 48 hours before admission, patient with brain hypoxia and brain trauma, patients in whom performing lumber puncture was contraindicated and patients who did not give consent were excluded from the study.

Laboratory method: Standard methods were used for the analysis and culture of CSF specimens collected from all suspected patients. Immediately after receipt, each CSF specimen was centrifuged at 1500 rpm for 15 minutes. The supernatant was removed and the sediment was cultured on 5% sheep blood agar and chocolate agar and MacConky's agar plates then incubated in a 5% CO_2 at 35°C for 48-72 hours. Gram staining was also performed. All isolates were identified based on their colony, morphology, culture characteristics, and biochemical reactions according to the standard microbiological procedures. Furthermore, cytological test and biochemical tests were done according to manufacturer's instruction (Protein & Glucose estimation by Flutitest USP, Analyticon, Germany). CSF was preserved at -70°C for DNA extraction. DNA was extracted according to Patho Gene-spin DNA extraction Kit, Intron Biotechnology). Primer sequence used for amplification was 5 / - T G A A G C G G A T T A T C A C T G G C - 3 / , 5/GCTAAACTCCCTGTATCAAGCG-3/.[10] Protocol of Thermal cycles of PCR for detection of lytA gene: Initial denaturation at 94°C for 3 minutes- 1 cycle, Denaturation at 92°C for 40 seconds, Primer annealing at 55°C for 30 seconds, Extension at 72°C for 20 seconds -35 cycles, Final extension at 72°C for 10 minutes-1 cycle.[11] Four microliters of the PCR reaction was loaded onto a 1.5% agarose gel containing ethidium bromide (0.5 mg/m) and gel electrophoresis was done for 20 minutes to separate PCR products. Presence of a 273-bp band under UV transilluminator was considered to be a positive.

Results

A total 272 clinically suspected meningitis cases were enrolled in this study from Chittagong Medical College Hospital (CMCH) .Table 1 shows categories of study population, according to cytological and biochemical findings, 68 (25%) were categorized as probable bacterial meningitis cases and 129 (47.22%) cases were viral meningitis, normal level of protein, glucose and cell count were found in 75 (27.78%) cases. Figure 1 shows, out of 68 probable bacterial meningitis cases, 17 (25%) were found positive by Gram stain, 22 (33.33%) cases were found positive by culture (Figure 2) and 27 (46.67%) cases were positive for S. pneumoniae by PCR for lytA gene among 57 probable bacterial meningitis cases (Figure 3). Table 2 Shows that among the 22 culture positive cases majority of the isolates were S. pneumoniae 11 (50%) followed by N. meningitidis 05 (23%), H. influenzae 3 (13.64%), E. coli 2 (9.1%) & S. aureus 1(4.54%). Table 3 shows comparison of culture with PCR by Chi-square test. The difference between culture and PCR to detect pneumococcal meningitis was statistically highly significant ($p < 0.01$). Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value were 100%, 65%, 41%, 100% respectively for lytA gene of S. pneumoniae by PCR considering culture as gold standard.

Table I : Showing categories of study samples according to cytological and biochemical (protein, glucose) findings

Biochemical & Cytological findings	Category	Frequency	Percentage (%)
Elevated Protein, Reduced Glucose, Neutrophilic pleocytosis> 100/ mm^3	Probable Bacterial meningitis	68	25
Protein elevated, Glucose level normal, Lymphocytic pleocytosis	Probable Viral meningitis	129	47.22
Protein level normal, Glucose level normal, Normal cell count	Normal	75	27.78
Total		144	100

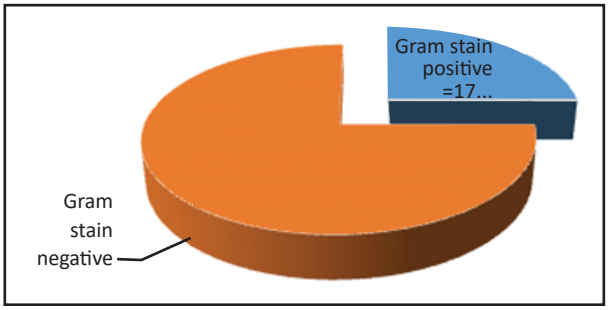

Figure 1: Results of Gram stain among the probable cases of bacterial meningitis

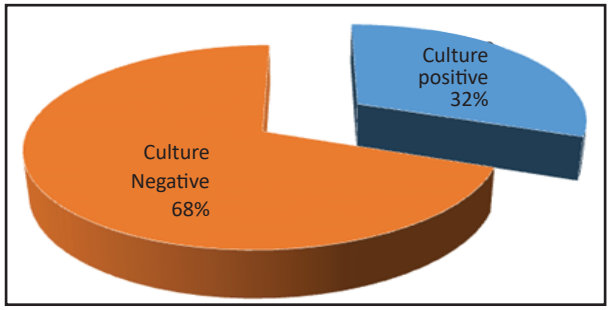

Figure 2: Results of culture among the probable cases of bacterial meningitis

Table II : Distribution of bacterial isolates among 22 CSF culture positive cases

Organism	Frequency	Percentage (%)
Streptococcus pneumoniae	11	50
Neisseria meningitidis	5	23
Haemophilus influenzae	3	13.64
Escherichia coli	2	9.1
Staphylococcus aureus	1	4.54
Total	22	100

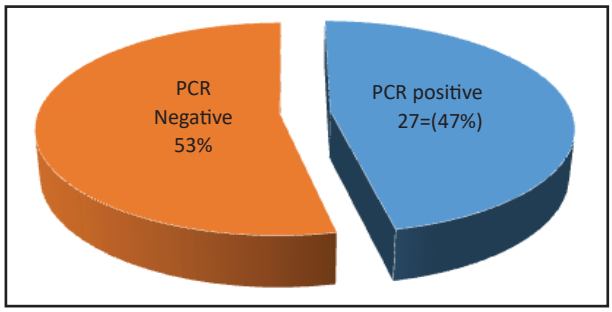

Figure 3: Results of PCR for lytA gene of S. pneumoniae among the probable cases of bacterial meningitis (n=57)

Table III : Comparison and evaluation of performance of PCR for detection of pneumococcal meningitis considering culture as gold standard

		Bacterial culture			Total
		Positive	Negative		
PCR	Positive	11	16	27	Sensitivity=100% Specificity=65%
	Negative	0	30	30	Positive predictive value(PPV) = 41% Negative predictive value(NPV) = 100%
Total		11	46	57	

χ2 Value= 8.57, p <0.01, highly significant

Discussion

Bacterial meningitis is still a very common and serious disease.[11] Globally 1.2 million cases of bacterial meningitis are estimated to occur every year with 135,000 deaths.[2] The case fatality rates (CFRs) in bacterial meningitis is 26% in developed countries even with antimicrobial therapy and availability of advanced intensive care which are higher ranging from 16-32% in developing countries.[12,13] On the basis of cytological and biochemical examinations of CSF, the study population was categorized into three groups. We found probable bacterial meningitis 68 (25%) cases, probable viral meningitis 129 (47.22%) cases and normal CSF 75 (27.78%) cases (Table 1). Negrini et al. (2000) had observed bacterial meningitis 20 (45%), aseptic meningitis 138 (64%) and non-meningitis group 18 (12.0%) cases.[14] Similarly, Narchi in Saudi Arabia (1997) observed in his study that 35 (35.7%) were bacterial meningitis and 63 (64.3%) were aseptic meningitis, which are comparable with the present study.[15] Figure 1 shows Gram stain provided an evidence of the causative bacteria in 17 (25.00%) cases which is similar to the observation by Yahia et al. 2014 (29.1%) but higher than that found by Saravalotz et al. 2003 (14.9%) & Schuurman et al. 2003 (9.31%) but much lower than that detected by Favaro et al. 2012 (75%).[7,16-18] The low yield of bacteria on gram stain can be explained by the facts that Gram stain depends on several factors like the number of pathogen present in the sample, prior use of antibiotics, technique used for smear preparation (cytospin centrifugation, direct smear etc.). In the present study, out of 68 probable cases of bacterial meningitis, 22 (32%) cases were positive by culture (Figure 2) which is similar to that found by Yahia et al. 2014 (34.5%).[16] Several studies showed culture negative cases of meningitis or a low CSF culture positivity ranging from 6 to 50% (Kabra et al.1991, Das et al. 2003, Chinchankar et al. 2002).[19-21] These variations of low yield of bacteria on culture may be due to antibiotic therapy prior to lumber puncture which is a common practice in developing countries. S. pneumoniae (50%) was the predominant organism followed by N. meningitidis (25%). H. influenzae (13.64%) E. coli (9.1%), S. aureus (4.54%) were

found (Table 2). Similar findings were observed by Reza et al. (2012) and Wellinder-Olson et al. (2007) who found S. pneumoniae was the predominant organism of bacterial meningitis.[22,23] In our study PCR analysis for lytA gene of S. pneumoniae detected 27 cases (47%) among 57 cases of probable bacterial meningitis(Figure 3). A similar study of PCR techniques conducted by Mashal Khan et al. (2013) quoted 39.15% and Yahia et al. (2014) picked 35.45% positivity by PCR.[16,24] For the evaluation of performance characteristics of PCR, result of PCR assay was compared with CSF culture as gold standard. According to this data Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of PCR for detection of Streptococcus pneumoniae were 100%, 65%, 41%, 100% respectively. Sensitivity of PCR was 100% (Table 3) which compare favorably with the results of Saravalotz et al. 2003 (100%) but not in good agreement with that found by Tzanakaki et al. (92.30%).17,25 Specificity (73.33%) was higher than Chiba et al. 2009 (54%), Sarookhani et al. 2013 (40.6%) but lower than Saravalotz et al. 2010 (98.2%).[17,26,27] However this specificity of PCR does not reflect the true percentage because in many cases with negative bacterial culture, an antibiotic had been prescribed before the bacterial cultivation of the CSF.[28–31]

Limitations: We used only one primer (lytA gene) from a number of primers, specific for Streptococcus pneumoniae and primers for other causative organisms were not included.

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Contribution of Authors: DTDR: Conception, acquisition of data, interpretation of data, drafting the article and final approval.

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Conclusion & Recommendation

Due to prior use of broad spectrum antibiotics conventional method may not yield the pathogen. This reemphasizes the need for molecular technique like PCR which is a highly sensitive, specific, rapid method and most importantly does not need the organism to be viable and can detect even when the microbial concentration is very low.

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