

COMPARATIVE STUDY OF POLYMERASE CHAIN REACTION AND ROUTINE MICROBIOLOGICAL METHODS FOR THE DIAGNOSIS OF PNEUMOCOCCAL MENINGITIS IN CSF

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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. This study was performed to compare between Polymerase Chain Reaction (PCR) and conventional methods for the diagnosis of pneumococcal meningitis. **Materials and Methods:** This cross sectional study was carried out in the Department of Microbiology, Chattogram Medical College for cytological examination, biochemical tests, Gram's stain, culture, and PCR for IytAgene of *S.pneumoniae* in CSF. **Results:** Among the 36 cases of probable bacterial meningitis, culture was positive in 12 (33.33%) and Gram's stain was positive in 9 (25%) cases. *Streptococcus pneumoniae* was the predominant organism detected by isolation in 6(50%). PCR detected 14(46.67%) cases of *S. pneumoniae* among 30 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%, 67%, 43%,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.

Key words

Pneumococcal meningitis; CSF; PCR.

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 diagnosis¹. Globally 1.2 million cases of bacterial meningitis are estimated to occur every year with 1,35000 deaths². The disease is much more common in developing countries than the developed countries. Gurley et al 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 child a year after introduction of the vaccine⁴. So except during an epidemic of meningococcal infection, *Streptococcus pneumoniae* is the commonest cause of acute bacterial meningitis⁵. 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

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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 agent⁶. But it requires a day or more for growth and can also give false negative result due to the preceding antibiotic therapy before admission or meningitis due to fastidious organisms¹. The increasing practice of preadmission administration of parenteral antibiotic therapy and reluctance to perform lumbar puncture at admission are pointed out to contribute a decrease in culture confirmed cases in several countries⁷. 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 meningitis⁸. PCR now can detect low number of pathogens in clinical specimens which does not require the presence of viable organisms⁹. So the purpose of the study was to determine the frequency of pneumococcal meningitis in different age group, to assess the diagnostic efficacy of PCR in identifying *lytA* gene of *Streptococcus*. This was the first study on bacterial meningitis to detect CSF bacterial pathogen by PCR in Chittagong Medical College and Hospital. To compare between Polymerase Chain Reaction (PCR) and conventional methods for the diagnosis of pneumococcal meningitis.

Materials and methods

A total of 144 clinically suspected patients of meningitis of age ranging from 0 day to 70 years from Neonatal, Paediatrics wards and Medicine wards of Chattogram Medical College Hospital (CMCH) and Chattogram Maa Shishu-O- General Hospital (CMSOGH) Chattogram were included in this study. This cross sectional descriptive study was carried out during the period of July 2015 to June 2016. Ethical clearance was duly obtained from Ethical Review Committee, Chattogram Medical College, Chattogram.

Inclusion criteria

Clinically suspected patients of meningitis with followings:

- i) Patients with high body temperature

- ii) Signs of meningeal irritation ie. neck rigidity, Kernig's sign, Brudzinski's sign

- iii) Headache

- iv) Vomiting

- v) Altered level of sensorium

- vi) High pitched crying

- vii) Photophobia.

Exclusion criteria

- i) Patients treated with injectable antibiotics for 48 hours before admission

- ii) Patient with brain hypoxia and brain trauma

- iii) Patients in whom performing lumbar puncture was contraindicated

- iv) Patients who did not give consent.

Standard methods were used for the analysis and culture of Cerebro Spinal Fluid (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% CO₂ 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 manufacturers instruction (Protein & Glucose estimation by Flutitest USP, Analyticon, Germany). CSF is 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'-TGAAGCGGATTATCACTGGC-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/ml) and gel electrophoresis was done for 20 minutes to separate PCR products. Presence of a 273-bp band under UV trans illuminator was considered to be a positive.

Results

A total 144 clinically suspected meningitis cases were enrolled in this study from Chattogram Medical College Hospital (CMCH) & Chattogram Maa Shishu -O- General Hospital. Table I shows categories of study population, according to cytological and biochemical findings, 36 (25%) were categorized as probable bacterial meningitis cases and 68(47.22%) cases were viral meningitis, normal level of protein, glucose and cell count were found in 40 (27.78%) cases. Fig-1 shows out of 36 probable bacterial meningitis cases, 9 (25%) were found positive by Gram stain, 12 (33.33%) cases were found positive by culture (Fig 2) and 14 (46.67%) cases were positive for S.pneumoniae by PCR for *lytA* gene among 30 probable bacterial meningitis cases (Fig 3). Table II shows that among the 12 culture positive cases majority of the isolates were *S. pneumoniae* 6(50%) followed by *N. meningitidis* 03(25%), *H. influenzae* 1(8.33%) *E. coli* 1(8.33%) & *S. aureus*1 (8.33%). Table III 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 100.00%, 67%, 43%, 100.00% respectively for *lytA* gene of *S. pneumoniae* by PCR considering culture as gold standard.

Table I : Categories of study sample according to cytological and biochemical (Protein, Glucose) findings (n=144)

Biochemical and Cytological findings	Category	Frequency	Percentage (%)
Elevated Protein, Reduced Glucose, Neutrophilic pleocytosis >100/ mm ³	Probable Bacterial meningitis	36	25
Protein elevated, Glucose level normal, Lymphocytic pleocytosis	Probable Viral meningitis	68	47.22
Protein level normal, Glucose level normal, Normal cell count	Normal	40	27.78
Total		144	100

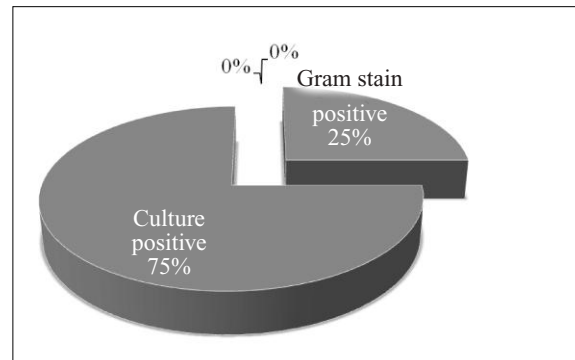


Fig 1 : Results of gram stain among the probable cases of bacterial meningitis

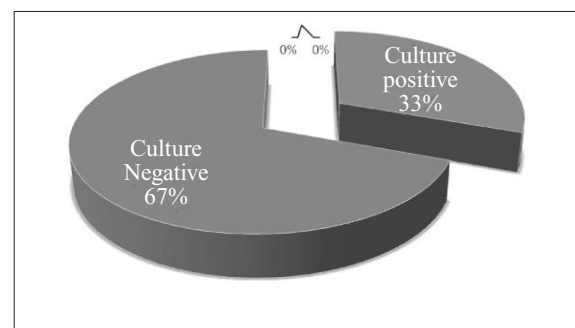


Fig 2 : Results of culture among the probable cases of bacterial meningitis

Table II : Distribution of bacterial isolates among 12 CSF culture positive cases

Organism	Frequency	Percentage (%)
<i>Streptococcus pneumoniae</i>	6	50
<i>Neisseria meningitidis</i>	3	25
<i>Haemophilus influenzae</i>	1	8.33
<i>Escherichia coli</i>	1	8.33
<i>Staphylococcus aureus</i>	1	8.33
Total	12	100

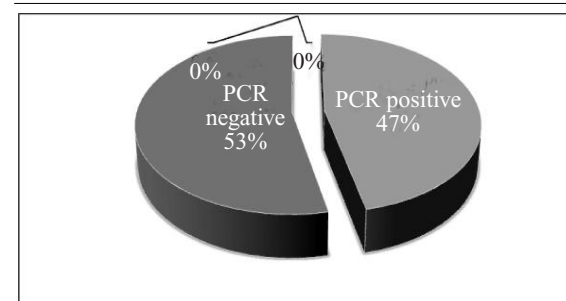


Fig 3 : Results of PCR for *lytA* gene of *S. pneumoniae* among the probable cases of bacterial meningitis

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

		Bacterial culture		Total	Sensitivity=100% Specificity=67%
		Positive	Negative		
PCR	Positive	6	8	14	Positive Predictive Value (PPV) = 43%
	Negative	0	16	16	
Total		6	24	30	Negative Predictive Value (NPV) = 100%

χ^2 Value=8.57, p <0.01, highly significant

Discussion

Bacterial meningitis is still a very common and serious disease¹¹. Globally 1.2 million cases of bacterial meningitis are estimated to occur every year with 135,000 deaths². 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 36(25%) cases, probable viral meningitis 68 (47.22%) cases and normal CSF 40 (27.78%) cases (Table I). Negri-niet al had observed bacterial meningitis 20 (45%), aseptic meningitis 138 (64%) and non-meningitis group 18 (12.0%) cases¹⁴. Similarly, Narchi in Saudi Arabia observed in his study that 35 (35.7%) were bacterial meningitis and 63 (64.3%) were aseptic meningitis, which are comparable with the present study¹⁵. Fig 1 shows gram stain provided an evidence of the causative bacteria in 9(25.00%) cases which is similar to the observation by Yahiaet al (29.1%) but higher than that found by Saravalotzet al (14.9%) & Schuurmanet al (9.31%) but much lower than that detected by Favaroet al (75%)^{7,16,17,18}. 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 36 probable cases of bacterial meningitis, 12 (33.33%) cases were positive by culture (Fig 2) which is similar to that found by Yahiaet al (34.5%)¹⁶. Several

studies showed culture negative cases of meningitis or a low CSF culture positivity ranging from 6 to 50% (Kabraet al, Das et al Chinchankaret al)^{19,20,21}. These variations of low yield of bacteria on culture may be due to antibiotic therapy prior to lumbar puncture which is a common practice in developing countries. *S.pneumoniae* (50%) was the predominant organism followed by *N.meningitidis* (25%). *H.influenzae*, *E.coli*, *S.aureus* were found 1 case each (Table-II). Similar findings were observed by Reza et al and Wellinder-Olson et al 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 14 cases (47%) among 30 cases of probable bacterial meningitis (Fig 3). A similar study of PCR techniques conducted by Mashal Khan et al quoted 39.15% and Yahiaet al 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%, 67%, 43%, 100% respectively. Sensitivity of PCR was 100% (Table III) which compare favorably with the results of Saravalotzet al (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 (54%), Sarookhani et al (40.6%) but lower than Saravalotzet al (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.

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.

Conclusion

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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Contribution of Authors

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

NA: Conception, data analysis, critical revision of content and final approval.

AKD: Conception, data analysis, critical revision of content and final approval.

SA: Design, data analysis, drafting, critical revision of content and final approval.

Disclosure

All the authors declared no conflict of interest.

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