

Identification of Etiological Agents of Pyogenic Meningitis by Gram Stain Culture in the Patients Admitted in Two Hospital

Diana Thecla D. Rozario^{1*}
Nasima Akter²
Arup Kanti Dewanjee³
Shakeel Ahmed⁴
Mohammed Majed⁵
Nahid Sultana⁶
Sanjoy Kanti Biswas⁶
Tanzina Nusrat⁶

¹Department of Microbiology
Abdul Malek Ukil Medical College
Noakhali, Bangladesh.

²Department of Microbiology
Chattogram Medical College
Chattogram, Bangladesh.

³Department of Microbiology
Marine City Medical College
Chattogram, Bangladesh.

⁴Department of Microbiology
Bangladesh Institute of Tropical & Infectious Diseases (BITID)
Chattogram, Bangladesh.

⁵Department of Microbiology
BGC Trust Medical College
Chattogram, Bangladesh.

⁶Department of Microbiology
Chattogram Maa-O-Shishu Hospital Medical College
Chattogram, Bangladesh.

*Correspondence to:

Dr. Diana Thecla D. Rozario
Assistant Professor
Department of Microbiology
Abdul Malek Ukil Medical College
Noakhali, Bangladesh.
Mobile : +88 01711 46 78 32
Email: dianadrozario17@yahoo.com

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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. The bacterial meningitis epidemiological landscape is not static and etiological agent varies with age and immune status and different geographic area. A simple Gram stained smear can offer immediate clues to aid the diagnosis of pyogenic meningitis. This study was performed to identify the causative agents of pyogenic 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. The gold standard for diagnosis of any infection including meningitis is the isolation and identification of the causative agent.

Results: Among the 144 suspected meningitis cases, 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. 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%).

Conclusion: *S. pneumoniae* is the predominant cause of pyogenic meningitis.

Key words: Pneumococcal meningitis; CSF; Pyogenic meningitis

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 2006¹. 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 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 the purpose of the study was to determine the trend of causative agent of pyogenic meningitis in patients admitted in Chattogram Medical College Hospital. This study was performed to identify the causative agents of pyogenic 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-O-Shishu Hospital (CMOSH) 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, i.e 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 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).

RESULTS

A total 144 clinically suspected meningitis cases were enrolled in this study from Chattogram Medical College Hospital (CMCH) & Chattogram Maa-O-Shishu Hospital. Table-I shows that highest cases of study population were in the age group of 1-<5 years 64(44.44%). The mean age of the patients was 10.11±14.22 years. Among the 144 cases, 82(57%) were male and 62(43%) were female where the male female ratio was 1.32:1. Table II 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-2 shows out of 36 probable bacterial meningitis cases 9 (25%) were found positive by Gramstain, 12 (33.33%) cases were found positive by culture (Fig 2). Table III 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 I : Age and Sex distribution of study population (n=144)

Age Group	Sex		Total
	Male	Female	
0-<1Months	1(0.69)	3(2.08)	4(2.78)
1m-<5 Years	41(28.47)	23(15.97)	64(44.44)
5- <18 Years	32(22.22)	28(19.44)	60(41.67)
>18 Years	8(5.55)	8(5.55)	16(11.11)
Total	82(57%)	62(43%)	144(100.00)

Male: Female=1:1.32, Mean age ±S.D=10.11±14.22

Table II : Categories of study sample according to cytological & biochemical (Protien, Glucose) findings (n=144)

Biochemical & 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

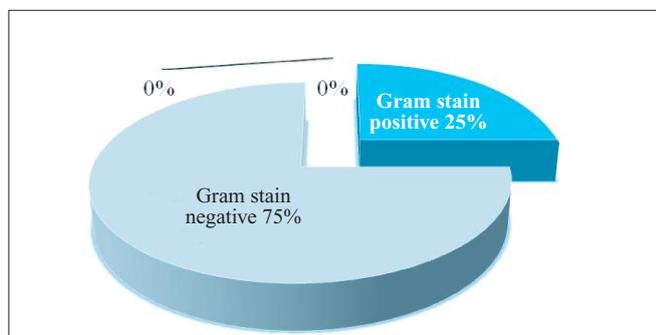


Figure 1 : Pie chart showing results of Gram stain among the probable cases of bacterial meningitis

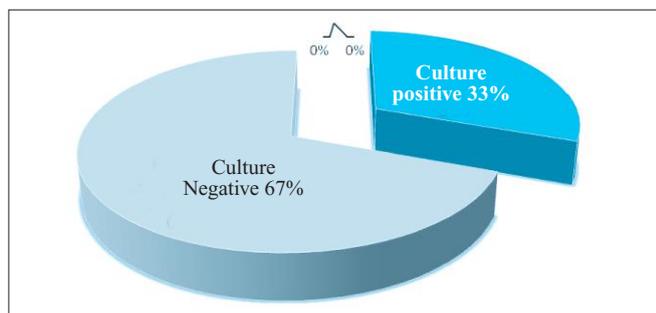


Figure 2 : Pie chart showing results of culture among the probable cases of bacterial meningitis

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

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

DISCUSSION

Bacterial meningitis is still a very common and serious disease¹. 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^{2,8}. In the present study majority of suspected cases of meningitis belonged to the age group of 1-<5 years 64(44.44%). 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 II). Negrinet al had observed bacterial meningitis 20 (45%), aseptic meningitis 138 (64%) and non-meningitis group 18 (12.0%) cases⁸. 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 study⁹. Fig-1 shows Gram stain provided an evidence of the causative bacteria in 9(25.00%) cases which is similar to the observation by Yahia et al (29.1%) but higher than that found by Saravalotz et al (14.9%) & Schuurmanet al (9.31%) but much lower than that detected by Favaro et al (75%)^{7,10,11,12}. 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 which is similar to that found by Yahia et al (34.5%)¹⁰. Several studies showed culture negative cases of meningitis or a low CSF culture positivity ranging from 6 to 50%^{13,14,15}. 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 III). Similar findings were observed by Reza et al and Wellinder-Olson et al who found *S. pneumoniae* was the predominant organism of bacterial meningitis^{16,17}.

CONCLUSION

Streptococcus pneumoniae is the commonest cause of acute bacterial meningitis

DISCLOSURE

All the authors declared no competing interest.

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