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LABORATORY-BASED CYTOLOGICAL AND BIOCHEMICAL PROFILE OF CEREBROSPINAL FLUID FOR CHILDREN WITH ACUTE BACTERIAL MENINGITIS IN BANGLADESH

MOHAMMAD ZAKERIN ABEDIN[1,3*], LAILA JARIN[2] AND DONALD JAMES GOMES[3]

[1]*Department of Microbiology, School of Biomedical Science, Khwaja Yunus Ali University, Sirajganj, Bangladesh.*

[2]*Department of Microbiology, Lab Aid Medical Centre Gulshan Ltd, Dhaka, Bangladesh.*

[3]*Department of Microbiology, University of Dhaka, Dhaka, Bangladesh.*

Abstract

An attempt was made to analyze the cerebrospinal fluid (CSF) for a cytological and biochemical profile to identify etiological agents from children with suspected acute bacterial meningitis. The cerebrospinal fluid (CSF) samples from 371 suspected meningitis cases were examined, and the highest bacterial meningitis was found in 52(14.0%) cases in this study. Among a total of 371 samples of CSF, 272(73.3%) were crystal clear, 52(14.0%) were moderately turbid, 47(12.7%) highly turbid. The total leukocyte cell count of the CSF was proportionate to the turbidity. In the case of crystal clear CSF's, total leukocyte counts (TLC) were normally ranging from <5 to 45 per mm^3 with predominant lymphocytes. Moderately turbid fluid showed 46 to 500 cells per mm^3 and highly turbid fluid showed from 501 to more than 10,00 cells/mm^3. In the latter cases, differential counts demonstrated polymorphonuclear predominance. In addition, about 100% (52 cases) of positive and 12.5% (40/319) of negative cases had CSF protein concentration >100 mg/dL. CSF protein concentration greater than 100 mg/dl and sugar level below 40 mg/dl were considered as suspected bacterial meningitis in this study. Surprisingly, the C-Reactive Protein (CRP) values were found to be >40 mg/dL in both culturally positive and negative cases. Most of meningitis positive cases showed increased total cell counts as well as protein concentration, and decreased serum sugar concentrations.

Keywords: Cytological patterns, Biochemical profile, Acute bacterial meningitis

Introduction

Bacterial meningitis continues to be a significant cause of mortality and morbidity in neonates and children throughout the world. Casualty in both developed and developing

*Corresponding author: <zakerin.du2016@gmail.com>.

countries of bacterial meningitis range from 4.5% and 15-50%, respectively. The percentage of mortality cases are near 100% in untreated people, and up to 40% of youngsters get fitting anti-infection treatment in developing nations (Salih *et al.* 1991).

After admission to the medical clinics, all happens within 72 hours; CSF protects the cerebrum from the sudden change in stable substance, pressure, and also maintains it and expels cerebral metabolism waste materials (McGing and Kelley 2009). CSF is the most significant part of evaluation for the laboratory diagnosis of meningitis. Bacteria, mycobacteria, and fungal diseases are created from CSF variations that may incredibly encourage conclusion and direct introductory treatment. All patients with acute bacterial meningitis ought to be performed the fundamental investigations of CSF include cell count, measurement of pressure, differential count of WBC, glucose, protein levels, Gram's stain properties, and cultural characteristics (Greenlee *et al.* 2009).

The composition of CSFs is liable for trademark changes in the meninges of meningitis patients by the infection of different pathogens that leads to changes in physiological and anatomical conditions of meninges. The expanded relocation of polymorphonuclear (PMN) leukocytes and leakage of protein into the CSF due to loss of trustworthiness of cerebral vessels and integrity of the blood-brain barrier. The standard CSF value is 0-5 leukocytes/mm^3, for the most part lymphocytes, however, in neonates, cell check is up to 30/mm^3 (Collee *et al.* 1996). A preponderance of neutrophils (WBC >500/mm^3) and monocytes (WBC >100/mm^3) is normal for a bacterial and viral meningitis respectively that is an impressive example cover is frequently found (Venkatesh *et al.* 2000).

Recognize bacterial meningitis (glucose <40 mg/dl) from aseptic meningitis (glucose are normally unaltered) by the level of CSF glucose (Mace 2008). The physiological activity of the choroid epithelium and utilization by bacterial pathogens and leukocytes leads to diminished CSF glucose (Watson *et al.* 1995). The count of white blood cells (>7500 cells/mm^3) and estimations of glucose (<10 mg/dL) levels are separated from chemical meningitis (Forgacs *et al.* 2001).

The blood-CSF barrier of CSF excludes a large amount of proteins. Protein accessing the CSF essentially arrives at the CSF by transport inside pinocytotic vesicles navigating delicate endothelial cells (McGing and Kelley 2009). The proteins level is more than 200 mg/dL of profoundly critical for acute bacterial meningitis showing interruption of the blood-cerebrum or the blood-CSF boundary (Mace 2008). In this investigation, we planned to study the profiling of cytological and biochemical parameters from cerebrospinal fluid from suspected bacterial meningitis in children in Bangladesh.

Material and Methods

Study Population: The study was done for one year, from August 2010 to August 2011. The 371 individuals with suspected meningitis in children were included. The children were clinically diagnosed with bacterial meningitis by physicians.

Sampling Sites: Samples were collected from different hospitals and diagnostic centers located in Dhaka city, Bangladesh, including Popular Diagnostic Center Ltd. and Central Hospital Ltd. After collection, the physical appearances of the CSF were noted. They were grouped according to their turbidity as crystal clear, moderately turbid, and highly turbid CSF. The cerebrospinal fluids (CSFs) were processed within one hour of gathering in the research facility. The samples were handled utilizing Gram staining, cell count, and Latex Agglutination Test (LAT) and biochemical analyses.

Analysis of TLC and DLC: In this study, a Neubauer counting chamber method and Wright's staining were used for cytological analysis of total leucocytes count (TLC), and differential leukocytes count (DLC) was done on the CSF specimen before centrifugation of the samples. Greater than 500 cells/mm^3 was significant for bacterial meningitis.

Estimation of CSF Protein and Glucose: CSF protein and glucose concentrations were determined by using the Vistors-250 System (USA). CSF protein concentration greater than 100 mg/dl and sugar level below 40 mg/dl were considered as suspected bacterial meningitis in this study.

Estimation of C-Reactive Protein (CRP): Cerebrospinal spinal fluids CRP level was estimated by using CRP latex agglutination kit (Chrono Lab, UK). On the provided slide, a drop of CSF was mixed with latex CRP reagent, and agglutination reaction was seen after 2 minutes. The presence of agglutination was considered a positive test. CRP level >40 mg/dl in CSF was taken as significant.

Latex Agglutination Test (LAT): The LAT was performed to identify the bacterial antigens of *Haemophilus influenzae* (HI), *Streptococcus pneumoniae* (SPN), *Neisseria meningitidis* (NM), and *Escherichia coli* (EC) using a Wellcogen Test- card latex agglutination (Thermo Fisher Scientific, USA) according to manufacturer's guidelines.

Data Analysis: The data obtained were analyzed by SPSS version 20 and Excel 2016. Descriptive statistics and chi-square tests were done to check the statistical significance.

Results and Discussion

Cerebrospinal fluid samples (n = 371) were aseptically drawn from different children with associated cases of bacterial meningitis patients aged between 2 months and 12

years from different hospitals and diagnostic centers in Dhaka city, Bangladesh. Most of them came from low income families. They were the victims of malnutrition and were suffering from several different diseases due to deficiencies of vitamins. Among the 371 patients, 63% (234/371) were male, and 37% (137/371) were females. Considering the positive cases, there was a slight predominance of males than females with a ratio of 1.7:1.

Latex Agglutination Test: Depending on the meningeal pathogen, latex agglutination has shown good sensitivity in detecting the antigens of common meningeal pathogens. The Latex agglutination test (LAT) method is one of the rapid tests for analysis of bacterial meningitis from CSF specimens. In this investigation, the LATs were positive for 14.0% (52/371) of CSFs (Table 1). In this LAT analysis, 50% (26/52) was *Streptococcus pneumoniae* followed by *Haemophilus influenzae* type b 21.1% (11/52), *Neisseria meningitides* 15.4% (8/52), and least of *Escherichia coli* K1 13.5 (7/52), that has also been observed by other studies (Shrestha *et al.* 2015).

Table 1. Latex agglutination test results from CSF by Wellcogen test- card.

Detected bacterial pathogens	Number of isolates	Percent
Streptococcus pneumoniae	26	50.0%
Haemophilusinfluenzae type b	11	21.1%
Neisseria meningitides	8	15.4%
Escherichia coli K1	7	13.5%
Total	52	100%

*Gr**am Staining Technique**:* The precipitate of a CSF sample is used for microscopy using Gram staining reaction for the preliminary identification of the organism. After staining, bacteria were found in 48 (12.9%) cases. No Gram- positive rod bacteria were found in any sample. The microorganisms found were Gram-negative cocci, Gram negative coccobacilli, Gram- positive cocci, and Gram -negative bacilli (rod- shaped) (Table 2).

Table 2. Morphology and staining properties of bacteria found in the CSF.

Morphology	Staining property	Number of isolates	Frequency (%)
Cocci (*Streptococcus pneumoniae*)	Gram- Positive	23	48.0
Coccobacilli *(Haemophilus influenzae type b)*	Gram -Negative	10	20.8
Cocci (*Neisseria meningitides)*	Gram -Negative	8	16.6
Rods (Escherichia coli)	Gram -Negative	7	14.6
Total		48	100%

Cytological Profile: The total numbers of leucocytes in the cerebrospinal fluid were counted as soon as the samples were brought to the laboratory. The crystal clear CSF showed 0-45 cell/$mm^3$and moderately turbid CSF showed 46-500 cells/mm^3. The highly turbid CSF was collected from cases of acute bacterial meningitis. In the latter cases, as great increases in the number of leucocytes were found with the range of 501 to >1000 cells/mm^3.The total number of leucocytes counted from different CSF is given in table (Table 3).

Table 3. Total leucocyte Cells (TLC) and positive cases (N=52) within analyzed samples.

	Total CSF samples (N=371)		Positive cases (N=52)	
TLC (cells/mm^3)	Frequency (N)	Percentage (%)	Frequency (N)	Positive rate (%)
<5-45	272	73.3	2	3.85
46-500	52	14.0	9	17.30
501- >1000	47	12.7	41	78.85

Most meningitis- positive cases showed increased total leuckocyte cell counts (TLC) and proteins and decreased serum sugar concentration. 78.85 % (41/52) of positive cases showed that TLC was higher than 501/mm^3, but 17.3% (9/52) and 3.85.0% (2/52) of positive cases showed that TLC was higher than 46/mm^3, and less than 45/mm^3, respectively. 73.30% (272 cases) showed that TLC was 0-45/mm^3, and within the cases, only 2 cases showed positive meningitis patients (Fig. 1).

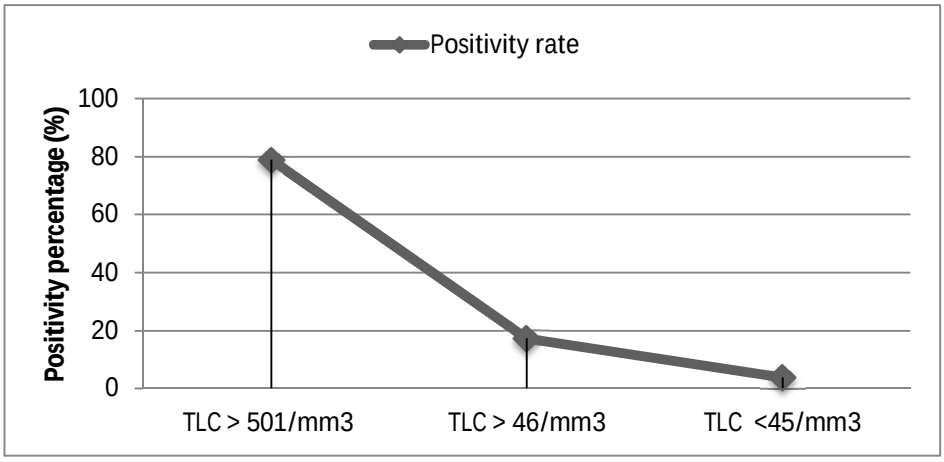

Fig. 1. Percentage of growth and TLC (Total Leukocyte cell) between TLC 0-100/mm^3 and TLC >100/mm^3

In our analysis, 41/52 of cloudy CSFs are affirmed acute bacterial meningitis cases. A few (2 cases) CSF is colorless, and only 9 samples of CSF are moderate turbid, additionally positive for bacterial meningitis. The color of the CSF to demonstrate infection could be deceiving, focusing on the need to utilize additional research facility indicative strategies. It is commonly acknowledged that WBC count >1000/mm^3 is responsible for an individual of bacterial meningitis.

The WBC ≥ 100/ mm^3 of the CSF is a more noteworthy rate were affirmed as bacterial meningitis individuals, which is steady with the standard portrayal of speculated bacterial contamination and, along these lines, a helpful trademark to survey the nearness of bacterial meningitis where culture offices are not accessible (Almeida *et al.* 2019).

In our study, total leukocyte count (TLC), protein and glucose more prominent than the normal range of 10 to >1000 cells/mm^3, 315 mg/dL, and 22.5 mg/dL, respectively, and with overwhelming neutrophils are observed among the 52 positive bacterial meningitis cases as observed by Pandey *et al.* (2015). Most studies, particularly expanded all out protein, increased white blood cells with neutrophils, and decreased glucose from CSF, and the nearness of countless PMN leukocytes and Gram staining is performed for microscopic organisms from CSF sediment that results in acute bacterial meningitis. Similar studies were also observed by other researchers (Dubos *et al.* 2008).

The total count of cells, glucose, total protein, and lactate levels were studied in cerebrospinal fluids. The pleocytosis of primarily polymorphic leukocytes is mainly due to bacterial meningitis occurring exemplary anomalies of CSF arrangement include low CSF to blood glucose proportion, low glucose focus, and raised protein levels. These variations are consistently missing within neonates. At 6% of cases in CSF with *S. agalactiae* bacterial meningitis were investigated within one hundred forty-six children (Johansson *et al.* 2015).

The CSF white blood cells tally was low, and the median was 6 cells/mm^3 and a territory amount of 0–90 000/mm^3, interquartile range of 2–15/mm^3. In the microbiologically confirmatory analysis of bacterial meningitis, CSF white blood cells tallies of more than 21 cells/ mm^3 had an affectability of 79% and explicitness of 81%. The concentrations of CSF glucose and protein varied from 0 to 198 mg/dL (median, 20 mg/dL) and 0.4 to 19.6 g/L (median, 2.7 g/L), respectively; microbiological culture-demonstrated bacterial meningitis was not analyzed precisely by cerebrospinal fluids protein or glucose (Alamarat *et al.* 2020; Johansson *et al.* 2015 and Panuganti 2017).

The CSF protein lower limits >0.5 g/L and total leukocyte count of >100 cells/mm^3 were additionally emphatically connected with acute bacterial meningitis patients were shown within a prospective investigation of 198 offspring of whom 98 had bacterial meningitis patients (Dubos *et al.* 2008). Another planned microbiological cultural investigation of CSF for meningococcal meningitis had a WBC count of not more than 1000 cells/mm^3 (19%) and an ordinary composition of CSF only 5 (1.7%) (Alamarat *et al*. 2020).

Biochemical Profiling of the CSF

Protein Concentration: In this study, 100% (52) of positive cases showed that protein was more than 100 mg/dL, while 75.2% (240/319) and 22% (70/319) of the negative cases had a protein level under 45 mg/dL and 100 mg/dL, respectively and 2.8% (09/319) of the negative case patients had a protein level higher than 100 mg/dL (Fig. 2). The mean CSF protein levels in the positive group were 315 mg/dL, more than the 117 mg/dL of the negative group.

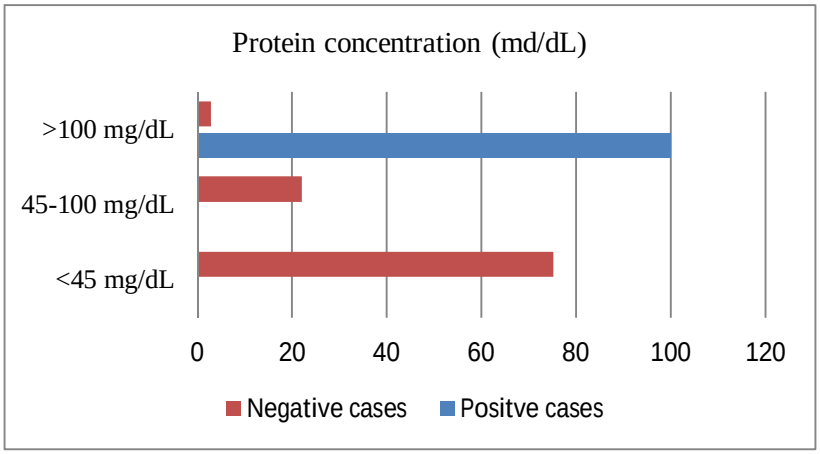

Fig. 2. Distribution of protein concentration between positive and negative cases suspected of acute bacterial meningitis in children.

Glucose Concentration: CSF glucose levels were also estimated in this study. CSF glucose levels at <40 mg/dL were found in about 94.2% (49/52) of 52 positive cases children, and only 5.8% (3/52) of positive cases had normal levels. Among the negative cases, CSF glucose levels at <40 mg/dL were found in only about 11.6% (37/319) of cases, while the glucose levels at 40 mg/dL were found in 88.4% (282/319) case samples (Fig. 3). In our study, 22.5 mg/dL and 53.3 mg/dl were the mean glucose level of bacterial meningitis positive cases and non-bacterial meningitis group, respectively.

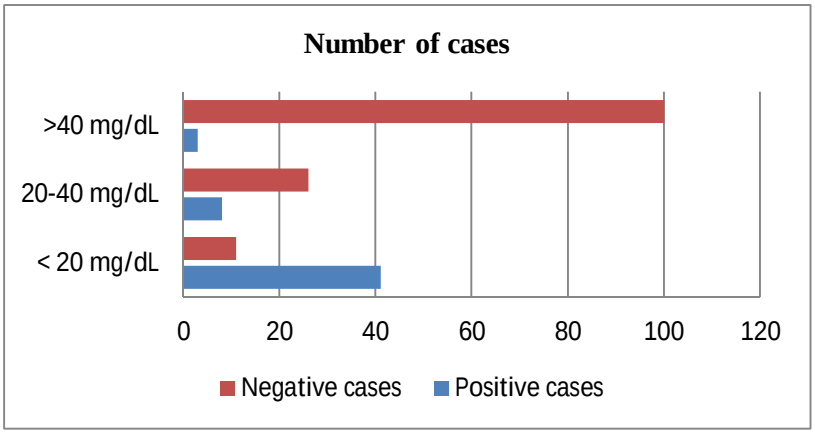

Fig. 3. Distribution of glucose concentration between positive and negative cases with suspected meningitis in children.

***C-Reactive Protein (CRP)*:** The amount of C-reactive protein (CRP) among culturally positive and negative suspected meningitis cases. CRP levels were high (>40 mg/dl) among 86.5% (45/52) of the positive bacterial meningitis children and only 24.8% (79/319) children with suspected non- bacterial meningitis had positive CRP tests. Only 13.4% (7/52) of the positive cases exhibited a negative CRP level, while 75.2% (240/319) of negative cases were also had negative CRP value.

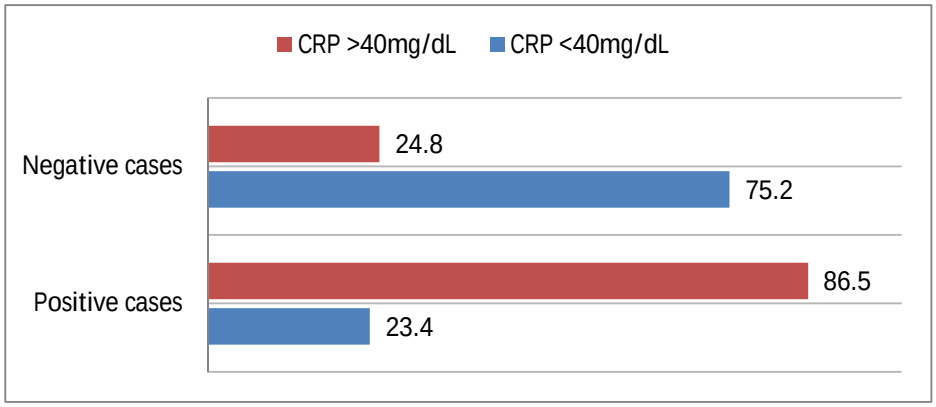

Fig. 4. C-Reactive Protein (CRP) levels are positive and negative in suspected meningitis cases.

In this study, the CSF protein value (>89.4 mg/dl) was higher than on average in bacterial meningitis cases whereas glucose estimation level (<28.8 mg/dl) was seen as diminished as normal range in meningitis patients (Table 4). The lab assumes a significant job in recognizing and affirming of meningitis cases. Assessment of the cerebrospinal liquid (CSF) is oftentimes used to give prompt affirmation of acute bacterial meningitis.

The amount of protein is expanded within the bacterial disease because the protein is discharged and microbial physiology within cerebrospinal fluids. Recognizing viral infection from bacterial meningitis can bring glucose and protein levels to normal value. The protein level is typically brought up in bacterial disease, and the glucose level is greatly low. If there is an occurrence of viral contamination, the protein value of remains practically typical. In this manner, this finding well with the built-up clinical information.

Conclusion

In this study, we have performed a cytological and biochemical analysis of CSF from acute bacterial meningitis of child patients in Bangladesh. The cytological characterization revealed 78.5% (41/371) positive meningitis patients when the TLC counts were more than 501cells/mm^3. Acute bacterial meningitis is depicted by increased total leucocytes cells (TLC) with amazing quality of neutrophils and reduced glucose and extended protein levels. The biochemical analysis of CSF showed >100 mg/dL of CSF protein. Most of the meningitis positive cases showed increased total cell counts as well as protein concentration, and decreased serum sugar concentrations. The most commonly observed pathogens in CSF latex agglutination test including *Streptococcus pneumoniae* (26, 50.0%), *H. influenzae* (14, 21.1%), *N. meningitides* (8, 15.4%), and *E.coli* K1 (7, 13.5%) as the causative bacterial pathogens.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

Almeida, S.M.D., N.C. Barros, R. Petterle and K. Nogueira. 2019. Comparison of cerebrospinal fluid lactate with physical, cytological, and other biochemical characteristics as prognostic factors in acute bacterial meningitis. Arq. Neuropsiquiatr. **77**(12): 871-880. https://doi.org/ 10.1590/ 0004-282X20190185

Alamarat, Zain, and Rodrigo Hasbun. 2020. Management of Acute Bacterial Meningitis in Children. *Infection and Drug Resistance.* **13**: 4077-4089.

Collee, J.G., J.P. David, A.G. Fraser, B.P. Marnionand, S.A. Simmon 1996. Laboratory strategy in the diagnosis of infective Syndromes. *In Mackie and McCartney Practical Medical Microbiology*. 14th ed. New York: Churchill-Livingstone. pp. 77-80

Dubos, F., B. Korczowski, D.A. Aygun, *et al*. 2008. Serum procalcitonin level and other biological markers to distinguish between bacterial and aseptic meningitis in children: a European multicenter case cohort study. *Arch Pediatr. Adolesc. Med.* **162**: 1157-63.

Forgacs, P., C.A. Geyer and S.R. Freidberg 2001. Characterization of chemical meningitis after neurological surgery. *Clin. Infect Dis.* **32**(2): 179-85.

Greenlee, J.E. 2009. Approach to diagnosis of meningitis. Cerebrospinal fluid evaluation. *Infect Dis Clin. North Am.* **4**(4): 583-98.

Johansson, K.U.N.D., M. Borgströmand S.A. Silfverdal. 2015. The clinical presentation of acute bacterial meningitis varies with age, sex and duration of illness. *Acta Paediatr*. **104**(11): 1117-1124.

McGing, P. and R.O. Kelley. 2009. The biochemistry of body fluids. Ireland: The Scientific Committee of the Association of Clinical Biochemists in Ireland (ACBI).

Mace, S.E. 2008. Acute bacterial meningitis. *Emerg. Med.Clin. North Am*. **26**(2): 281-317.

Pandey, P., B. Jha and A. Shrestha. 2015. Cytological and Biochemical Profile of Cerebrospinal Fluid from Meningitis Patients. *Nepalese Association for Cl. Chem, ACCLM*. **1**(1): 2-5

Panuganti, S.K.N.S. 2017. Acute bacterial meningitis beyond the neonatal period. In: Long SSPC, Fischer M, editors. *Principles and Practice of Pediatric Infectious Diseases*. 5th ed. Elsevier. 278-287.

Shrestha, R.G., S. Tandukar, S. Ansari, *et al.* 2015.Bacterial meningitis in children under 15 years of age in Nepal. *BMC Pediatr.* 15, 94. https://doi.org/10.1186/s12887-015-0416-6]

Salih, M.A.M., O.H. Khaleefa, M Bushara, *et al.* 1991. Long term sequelae of childhood acute bacterial meningitis in a developing country. *Scand. J. Infect. Dis.* **23**: 175-82.

Venkatesh, B., P. Scott and M. Ziegenfuss. 2000. Cerebrospinal fluid in critical illness. *Crit. Care Resusc.* **2**: 42-54.

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