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Research Article

LIMITATIONS OF NORMAL CSF CELL COUNTS IN EXCLUDING BACTERIAL MENINGITIS: A MULTICENTRIC HOSPITAL BASED STUDY IN KATHMANDU, NEPAL

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ABSTRACT

An increase in cerebrospinal fluid (CSF) cell count is an indicator of the diagnosis of bacterial meningitis. However, studies have reported that few bacterial meningitis cases showed no abnormalities in initial CSF analysis. Therefore, this study aimed to analyze the CSF cell count in culture positive bacterial meningitis cases. A cross-sectional hospital based prospective study was conducted from January 2017 to December 2018 among 387 CSF samples collected from clinically suspected meningitis cases attending different hospitals located in Kathmandu, Nepal. Each sample was processed for bacterial culture, total and differential leucocyte count, and protein and glucose concentration determination. Among the total CSF specimens (n=387), 32 (8.27%) were positive by culture for bacterial isolates. Bacteria were isolated from more number of CSF samples with pleocytosis, increased protein, and decreased glucose concentration. However, four meningococcal and two pneumococcal cases had normal CSF cell count (0-5 cells/mm^3), protein (15-45 mg/dl), and glucose (45-80 mg/dl) concentration. Normal CSF cell count cannot always rule out bacterial meningitis. Therefore, diagnosis shouldn't rely solely on cell count and CSF culture should also be considered.

Keywords: Bacterial meningitis, CSF Cell count, Nepal, pleocytosis

INTRODUCTION

Bacterial meningitis is a life-threatening infection of the meninges, characterized by high mortality and morbidity rates if not diagnosed and treated promptly. Globally, it affects millions annually, with significant prevalence in low- and middle-income countries, including Nepal. Clinical symptoms of bacterial meningitis typically include fever, headache, neck stiffness, altered mental status, and vomiting, which can overlap with other forms of meningitis or central nervous system infections. Differentiation of bacterial from other meningitis is important for deciding the treatment (Sharma et al., 2020). Early diagnosis of bacterial meningitis is crucial for the effective treatment of cases (Gordon et al., 2017). Since the rate of culture positivity is quite low, diagnosis in developing countries like Nepal relies on interpretation of biochemical and cytological parameters of cerebrospinal fluid (CSF). Typical abnormalities in CSF associated with bacterial meningitis include pleocytosis with predominant polymorphonuclear (PMN) leucocytes, decreased glucose concentration and increased protein concentration (WHO, 2011). Literature suggests the higher diagnostic ability of CSF cell count for bacterial meningitis compared to CSF protein, glucose, lactate dehydrogenase or chloride especially in case of neonates (Huang et al., 2019).

The normal cytology of CSF is 0-5 white blood cell (WBC)/mm^3 in adults and 10-30 WBC/mm^3 (50% PMNs) in infants (Erdem et al., 2017; WHO, 2011). In case of untreated bacterial meningitis, WBC count is increased to 1000–5000 cells/mm^3 with predominance of neutrophils (Tunkel et al., 2004). Therefore, pleocytosis seems to be important in establishing the diagnosis of meningitis. However, previous studies report that few bacterial meningitis cases did not display abnormalities in initial CSF analysis (Erdem et al., 2017; Jolobe, 2017). Absence of pleocytosis represents a diagnostic challenge since bacterial meningitis might be missed in such circumstances. Hence, this study aimed to analyze the CSF cell count and glucose and protein concentration in culture positive bacterial meningitis cases.

MATERIALS AND METHODS

Study design

This cross-sectional prospective study was conducted from January 2017 to December 2018 among 387 clinically suspected meningitis cases attending different hospitals located in Kathmandu, Nepal. The study sites were the major referral hospitals within Kathmandu valley of Nepal which included Bhaktapur Hospital, Bir

hospital, Kanti Children's Hospital (KCH), Sukraraj Tropical and Infectious Diseases Hospital (STIDH) and Tribhuvan University Teaching Hospital (TUTH). The details on study sites have been described elsewhere (Sharma et al., 2019a).

Ethics approval and consent to participate

Ethical approval of this study was obtained from Nepal Health Research Council (Reg. No. 465/2016). At the time of enrollment, written informed consent was taken from the patients or their caregivers/ guardians on behalf of the patients. Parents or guardians were assured about the non-disclosure of information collected from them and were also informed about the use of data for analysis and using the results for improving patient care activities as well as publication without disclosing the name or identity of cases. This study was conducted in accordance with the Declaration of Helsinki.

Sample collection and processing

CSF sample was collected from each clinically suspected meningitis case by the concerned physician/medical officer by lumbar puncture at the respective study site. Each sample was processed for determination of cytological and biochemical parameters.

Cell count

Each CSF sample was examined for total leucocyte count (TLC) by using Neubauer counting chamber. Briefly, one drop of CSF was diluted with 19 drops of WBC diluting fluid (pH 2.2$\pm$0.2) composed of 2% glacial acetic acid which lysed the RBCs and 1% Gentian violet which stained the nuclei of leucocytes. The Neubauer counting chamber was assembled making sure the chamber and cover glass were completely clean. Using a fine bore Pasteur pipette, the counting chamber was carefully filled with the diluted CSF taking care not to overflow into the channels on each side of the chamber. After waiting for about 2 minutes for the cells to settle, the cells were observed microscopically under 10X and 40X objectives for polymorphonuclear neutrophils and lymphocytes. If a mixture of both, the percentage of each cell type was estimated. The number of cells per litre of CSF (N) was calculated and reported as

$$N = \frac{\text{Number of cells counted (n)} \times \text{Dilution factor}}{\text{Depth of fluid} \times \text{Area counted}} = \frac{n \times 20}{0.1 \times 4} = n \times 50$$

Differential leucocyte count (DLC) was done by using Wright stain.

CSF protein determination

CSF total protein (TP) concentration was determined by pyrogallol red method by using the fully automated analyzer (Erba XL-200 Germany) as per the manufacturer's instructions at two study sites. At other sites, protein concentration was estimated by turbidimetric method using commercially available kit. Briefly, 1500µl of reagent was pipetted to three test tubes labeled as blank (B), test (T) and standard (S). CSF sample (500µl) was added to T, standard solution (500µl) to S and distilled water (500µl) to B tube and mixed well. All the three tubes were then placed at water bath for ten minutes and absorbance of the test sample and standard were measured in colorimeter using 520 nm filter after setting the colorimeter at zero using blank. The protein concentration of the sample was calculated as

$$Protein\ (mg\ \%) = \frac{Absorbance\ of\ the\ test\ solution}{Absorbance\ of\ the\ standard\ solution} \times Concentration\ of\ standard$$

CSF glucose determination

CSF glucose concentration was determined by using the fully automated Erba XL-200 Germany as per the manufacturer's instructions at two study sites. At other study sites, glucose concentration was estimated by enzymatic method using the commercially available kit. Briefly, 2000µl of reagent was pipetted to three test tubes labeled as blank (B), test (T) and standard (S). CSF sample (20µl) was added to T, standard solution (100 mg %) (20µl) to S and distilled water (20µl) to B tube and mixed well. All the three tubes were then placed at water bath (37°C) for 5 minutes and absorbance of the standard and test sample were measured in colorimeter using 520 nm filter after setting the colorimeter at zero using blank. The glucose concentration of the sample was calculated as

$$Glucose\ (mg\ \%) = \frac{Absorbance\ of\ the\ test\ solution}{Absorbance\ of\ the\ standard\ solution} \times Concentration\ of\ standard$$

Culture of CSF samples

Each CSF sample was inoculated into blood agar and chocolate agar (Hi Media Laboratories, Pvt. Limited, India) and incubated in candle jar at 37ºC for 24 hours. The bacterial isolates were identified at National Public Health Laboratory (NPHL), Teku, Kathmandu by standard microbiological techniques such as observation of colony characteristics, Gram's staining, catalase test, oxidase test and other required biochemical tests (Tille,

2020). The details on culture have been described previously by Sharma et al. (2019a).

Data analysis

The obtained data was entered into Microsoft office Excel 2007 and exported into IBM Statistical Package for Social Sciences (SPSS) version 21 for analysis. Chi-square test was used to compare between groups and a p-value of <0.05 was considered to be statistically significant.

RESULTS

Among the total CSF specimens (n=387), 32 (8.27%) were positive by culture for bacterial isolates. Table 1 describes the cytological and biochemical parameters of CSF samples.

Table 1. Cytological and biochemical parameters of CSF samples (n=387)

Parameter	Range		Mean	
	Culture positive CSF samples (n=32)	Culture negative CSF samples (n=355)	Culture positive CSF samples (n=32)	Culture negative CSF samples (n=355)
TLC (cells/mm^3)	0-2000	0-1100	360	12.18
DLC (%)				
Lymphocytes	5-88	10-95	27.10	48.81
Neutrophils	12-95	5-90	72.81	51.19
Glucose (mg/dl)	1.5-70	3.50-112.00	36.51	53.88
Protein (mg/dl)	16-170	5.10-615.00	77.37	46.78

Isolation of bacteria was higher (41.27%) from CSF samples with TLC > 5 WBCs/mm^3 (Table 2).

Table 2. Association of culture results with TLC of CSF specimens

TLC (WBCs/mm^3)	Culture positive CSF samples n (%)	Culture negative CSF samples n (%)	p-value (calculated using chi-square test)
0-5†	6 (1.85)	318 (98.15)	χ^2=108.046, df=1,
> 5	26 (41.27)	37 (58.73)	p=0.000

† denotes normal range

Similarly, the isolation of bacteria was higher from CSF samples with protein level > 45 mg/dl and glucose level < 45 mg/dl (Table 3).

Table 3. Association of culture results with protein and glucose concentration of CSF specimens

Parameters	Culture positive CSF samples n (%)	Culture negative CSF samples n (%)	p-value (calculated using chi-square test)
Protein concentration (mg/dl)			χ^2=23.72, df=1, p=0.000
≤ 45†	9 (3.47)	250 (96.53)	
> 45	23 (17.97)	105 (82.03)	
Glucose concentration (mg/dl)			
< 45			χ^2=40.431,df=1,p=0.000
	24 (22.86)	81 (77.14)	
≥ 45†	8 (2.84)	274 (97.16)	

† denotes normal range

Four meningococcal and two pneumococcal cases had normal CSF cell count (0-5 cells/mm^3), total protein (15-45 mg/dl) and glucose (45-80 mg/dl) concentration but were detected by culture. Normal CSF cell counts were observed exclusively in children (7.50%) and adolescents (9.09%) who were culture-positive for bacterial meningitis. This highlights the potential diagnostic challenge in these age groups, as normal CSF cell counts may delay or mislead the diagnosis (Table 4).

Table 4. Age wise distribution of clinically suspected and bacterial culture positive meningitis cases with normal CSF cell count

Age group	**Clinically suspected meningitis cases n (%)**	**Culture positive meningitis cases n (%)**	**Normal CSF cell count n (%)**
Neonate (below 1 month)	128 (33.07)	13 (10.16)	0 (0.00)
Infant (1 month- below 1 year)	74 (19.12	4 (5.41)	0 (0.00)
Child (1- below 10 years)	40 (10.34)	6 (15.00)	3 (7.50)
Adolescent (10- below 19 years)	33 (8.53)	6 (18.18)	3 (9.09)
Adults (19-45 years)	80 (20.67)	1 (1.25)	0 (0.00)
Adults (above 45 years)	32 (8.27)	2 (6.27)	0 (0.00)

DISCUSSION

Culture is considered as gold standard for the diagnosis of bacterial meningitis. However, its diagnosis in developing countries like Nepal is based on CSF cell count, glucose and protein concentration due to low positivity of culture and Gram's staining results (Sharma et al., 2020, 2021, 2019b). Increase in CSF WBC count is considered as an indicator in the diagnosis of bacterial meningitis (WHO, 2011; Ye et al., 2016). In case of patients with bacterial meningitis, the permeability of blood-brain barrier is increased. Hence, neutrophils could cross the blood-brain barrier and migrate to the infection site leading to tissue damage (Doran et al., 2016). However, six CSF specimens in our study showed no WBCs on TLC. Bacterial meningitis in absence of pleocytosis was also reported by other researchers (Erdem et al., 2017; Fishbein et al., 1981; Hase et al., 2014; Jolobe, 2017). The culture positive cases without pleocytosis might be due to the fact that specimens were collected at an early stage of infection so that the bacteria were present, but no adequate inflammatory reaction occurred in the patients (Hase et al., 2014; Troendle & Pettigrew, 2019). This suggests that the normal CSF at an early stage cannot rule out bacterial meningitis. Our finding of pneumococci and meningococci on culture of CSF specimens without pleocytosis was similar to that described elsewhere (Hase et al., 2014; Troendle & Pettigrew, 2019). A low WBC count in CSF was also reported in case of sepsis in adults with pneumococcal meningitis (Weisfelt et al., 2006). Erdem et al reported CSF pleocytosis in 3% of tuberculous meningitis cases (Erdem et al., 2017). The timing between specimen collection and processing might play a role in the TLC results. Delays in CSF analysis can alter the cell count due to cell lysis as there is progressive reduction in both neutrophils and lymphocytes after 4 hours (Kestenbaum et al., 2010).

In meningitis, the disruption in the blood brain barrier leads to elevated CSF protein concentration. Hence, increased CSF protein concentration is also used as an indicator of bacterial meningitis (WHO, 2011).

Glucose is also frequently used as an indicator in the diagnosis of bacterial meningitis. Similar to our findings, more culture positive cases were detected among those with decreased CSF glucose by other researchers as well (Fouad et al., 2014). However, researchers have suggested that glucose concentration is not a stable parameter in neonates as it could be influenced by the administration of intravenous glucose while hospitalization (Huang et al., 2019). CSF glucose must be measured as soon as possible (within 20 minutes) after collection otherwise a false low result might be obtained due to glycolysis (Tille, 2020). Moreover, serum glucose should also be evaluated in the serum samples collected about 2 hours prior to spinal tapping. The absence of normal CSF cell counts in neonates and adults suggests that pleocytosis is a more consistent indicator in these groups. The presence of normal CSF cell counts in children and adolescents with culture-positive meningitis underscores the importance of not relying solely on CSF cell counts for diagnosis in these age groups. Complementary diagnostic methods, such as culture or molecular tests, are crucial. It is possible that these patients were in the early stages of bacterial meningitis when inflammation had not yet resulted in pleocytosis. This aligns with previous findings that normal CSF parameters can occur during the initial phase of infection. These findings highlight the importance of considering the clinical stage of infection when interpreting CSF results. For patients with positive cultures but normal CSF findings, early diagnosis through culture or molecular testing is critical to prevent delays in treatment, which could lead to adverse outcomes. This underscores the limitations of relying solely on CSF cytological analysis and the necessity for comprehensive diagnostic approaches. The strength of our study is that all age groups from multicentric sites have been included making the study population representative. The major limitation of our study is that sample size of culture positive cases is small and results may be difficult to generalize. Furthermore, being multicentric study, there might be some variations in the results due to different instruments being used in different settings. It is recommended to study a large number of samples to make a clear conclusion on CSF cell count and culture.

CONCLUSIONS

Normal CSF cell count cannot always rule out bacterial meningitis. Therefore, diagnosis shouldn’t rely solely on cell count and CSF culture should also be considered.

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AUTHOR CONTRIBUTIONS

SS, JA, BSC, RK, BKY, MRB, PG and AS designed the study. JT, SA, MK, RK and BSC collected the samples. DKK, PB and SS performed the laboratory investigations. SS reviewed the literature and drafted the manuscript. JA, BKY, BSC, MRB, PG and AS critically reviewed the manuscript. All authors read the manuscript and approved the final version.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICAL STATEMENT

Ethical approval of this study was obtained from Nepal Health Research Council (Reg. No. 465/2016). At the

time of enrollment, written informed consent was taken from the patients or their caregivers/ guardians on behalf of the patients. Parents or guardians were assured about the non-disclosure of information collected from them and were also informed about the use of data for analysis and using the results for improving patient care activities as well as publication without disclosing the name or identity of cases.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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