Original Article

Role of neonatal cerebrospinal fluid cytology in correlation to C-reactive protein, blood culture, risk factors and clinical outcomes in neonatal intensive care

Prita Pradhan[1], Reshmi Mishra[2], Santosh Kumar Panda[2], Ranjita Panigrahi[1], Urmila Senapati[1], Rajlaxmi Sarangi[3], Kumudini Panigrahi[4]

[1]Department of Pathology, Kalinga Institute of Medical Sciences, KIIT University, Bhubaneswar, Odisha, India, [2]Department of Pediatrics, Kalinga Institute of Medical Sciences, KIIT University, Bhubaneswar, Odisha, India, [3]Department of Biochemistry, Kalinga Institute of Medical Sciences, KIIT University, Bhubaneswar, Odisha, India, [4]Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT University, Bhubaneswar, Odisha, India

Abstract

Introduction: The number of neonatal cerebrospinal fluid (CSF) samples sent from the neonatal intensive care unit (NICU) for cytologic examination is rising, warranting accurate analysis and interpretation of the same. This study was taken up to assess the usefulness of CSF cell count and cytology in NICU settings, as it can be used even in a resource-limited setting. **Aim and Objective:** 1) To study the prevalence of cell count and cytologic changes in CSF from NICU and assess their usefulness in correlation to C-reactive protein, CSF neutrophil percentage, blood, CSF culture, and other biochemical parameters. 2) To correlate cell counts and cytology with age, period of gestation, presence, and absence of sepsis, seizures, intracranial hemorrhage, and their clinical follow-up. **Materials and Methods:** A retrospective study was done on neonatal CSF samples submitted for cytology over one year (January–December 2016) in the Department of Pathology. CSF cell counts were retrieved, and cytosmears were reviewed for cellularity, cell type, proportion, and background and correlated with the biochemical, microbiological, and clinicoradiological findings. **Results:** A total of 213 samples were included with 140 males and 73 females with an age range of 0–28 (mean: 7.3) days. The mean CSF cell count was 5.48/cu.mm (0–90 cells/cu.mm). The most frequent cytologic finding was occasional lymphocytes or acellular CSF (63.9%). The CSF leucocyte count and protein levels showed a significant correlation with s C-reactive protein. The CSF cytology showed a significant correlation between the age of the neonate and blood neutrophil percentage ($P = 0.0158$). History of intracranial hemorrhage showed a significantly higher frequency of the presence of red blood cells ($P = 0.0147$). **Conclusion:** Accurate cell counts, cytology of neonatal CSF, and biochemical and microbiological workup can help diagnose and manage neonates in intensive care.

Keywords: Hemorrhage, meningitis, preterm

Introduction

Neonatology is a vast expanding subject. So, the number of newborns admitted to the neonatal intensive care unit (NICU) and the neonatal cerebrospinal fluid (CSF) samples sent for cytologic examination is rising. It needs timely and accurate analysis and interpretation of the CSF analysis. In neonates, they pose great challenges regarding the procedure, the number of samples available for various investigations, and the technical possibilities. Cell counts in NICU can be performed on a quick bedside basis, while cytology requires cytocentrifugation and

Address for correspondence: Dr. Prita Pradhan, Assistant Professor, Department of Pathology, Kalinga Institute of Medical Sciences, Bhubaneswar - 751 024, Odisha, India.
E-mail: prita88@gmail.com

Received: 26-05-2021 **Revised:** 29-11-2022
Accepted: 16-12-2022 **Published:** 31-05-2023

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_980_21

How to cite this article: Pradhan P, Mishra R, Panda SK, Panigrahi R, Senapati U, Sarangi R, *et al*. Role of neonatal cerebrospinal fluid cytology in correlation to C-reactive protein, blood culture, risk factors and clinical outcomes in neonatal intensive care. J Family Med Prim Care 2023;12:932-9.

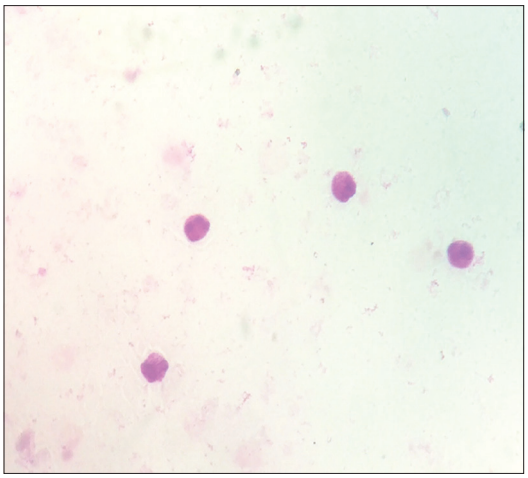


Figure 1: CSF of a neonate with no CNS abnormality showing occasional lymphocytes only (Leishman stain, 1000X)

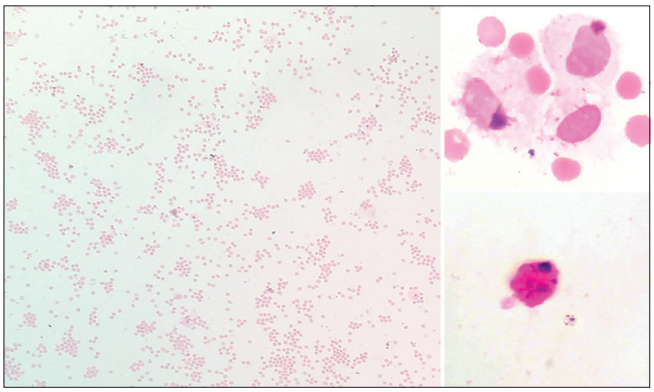


Figure 2: Smear showing numerous RBCs with upper inset showing few macrophages in aggregates. The lower inset highlights hemosiderin laden macrophages. (Leishman stain 40X; upper inset: Leishman stain 1000X and lower inset Perls stain, 1000X)

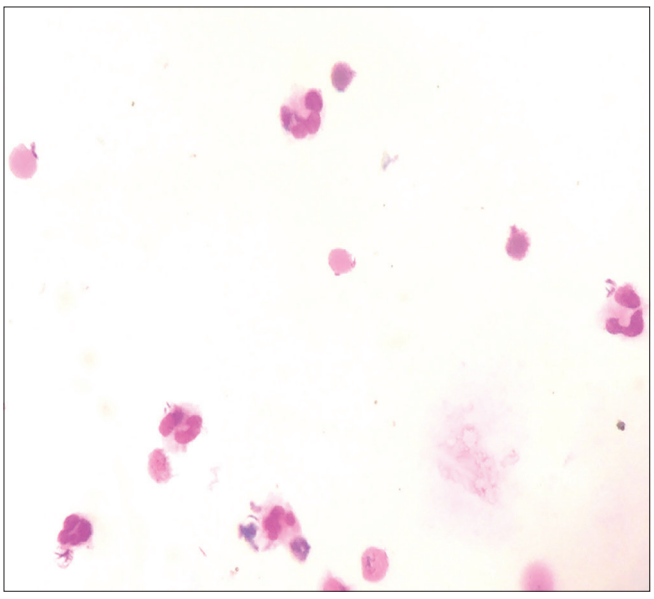


Figure 3: Cytocentrifuged CSF of a case of meningitis showing numerous neutrophils with few lymphocytes (Leishman stain, 1000X)

staining set-up, which requires the technical expertise of a good cytotechnologist and cytopathologist.[1]

The blood-brain barrier (BBB) of children is different from that of adults.[2,3] Neonates are often admitted with sepsis, respiratory distress, birth asphyxia, and neonatal seizures.[3] Equally important are those with risk factors for meningitis, particularly preterm neonates, which can result in neurodevelopmental issues on follow-up.[4] Hence, the indications for neonatal CSF cytologic assessment are diverse. The use of antibiotics in these babies, causing no growth on CSF culture, has increased the need for other diagnostic methods to clinch meningitis. A frequent query is whether the mere cell counts should suffice or is the CSF whether the CSF cytologic assessment in neonates is to add any further useful information. Also, can a simple CSF study suffice in case of paucity of access to other biochemical and microbiological tests in resource-limited settings? Furthermore, there are limited studies comparing the CSF findings of NICU neonates with those of healthy neonates. To address these questions, this study was done: 1) to study the prevalence of cell count and cytologic changes in CSF from NICU and assess their usefulness in correlation to C-reactive protein, CSF neutrophil percentage, blood, and CSF culture, and other biochemical parameters; 2) to correlate cell counts and cytology with age, period of gestation, presence and absence of sepsis, seizures, intracranial hemorrhage, and their clinical follow-up.

Materials and Methods

This was a hospital-based retrospective cross-sectional archival study on archival records performed while maintaining confidentiality in accordance with the standard institutional ethical guidelines in compliance with the Helsinki Declaration of 1964, as revised in 2013. All the consecutive neonatal CSF samples received from NICU over one year (January–December 2016) in the cytology section, Department of Pathology of a tertiary care centre, were included in the study. As the study focussed on diagnostic usefulness, insufficient samples and repeat samples on follow-up were excluded from the study. The relevant clinical details from the medical records were retrospectively extracted from the archives, including age at admission, sex, inborn or referred, singleton or multiple gestations, term/pre-/post-term, gestational age, type of delivery, and diagnosis at the time of admission and discharge.

CSF cell counts were extracted from the cytology records. The air-dried and fixed, stained cytocentrifuged smears of CSF were reviewed for cellularity, cell type, proportion, and background. The biochemical findings included were CSF glucose, protein and serum, highly sensitive C-reactive protein (CRP), and procalcitonin levels. The microbiological findings documented were CSF gram stain findings along with CSF, blood culture, urine, and other fluid/swab sent for culture. As indicators of sepsis, the total leucocyte count and neutrophil percentage in blood were noted. The CSF cell count and cytology were correlated with clinicoradiological, biochemical, and microbiological findings.

Subgroup analysis was done in 'term' versus 'preterm', 'sepsis' versus 'non-sepsis', and 'seizure present' versus 'seizure absent'. The findings in cases with intracranial hemorrhage and those with structural abnormalities were compared with those having no central nervous system (CNS) abnormalities on imaging. The Chi-square test was used for comparing the categorical variables, and the unpaired *t*-test was used for the comparison of continuous variables. Statistical analysis was done using Pad Prism 7 and MedCalc. *P* value of <0.05 was taken as significant.

Results

A total of 213 neonatal CSF samples satisfying the inclusion and exclusion criteria during the study period were studied.

Demographic data

The age range was from 0 to 28 days (mean age, 7.3 days), including 140 males and 73 females, with 70.4% of the babies in the first week of life. The cases referred from other centres for further treatment constituted 67%. The singleton pregnancy comprised 88%. The distribution of neonates based on maturity at birth and the different clinical diagnosis are shown in Graphs 1 and 2, respectively. The number of term neonates was comparable with the preterm. Sepsis was the most frequent clinical diagnosis at admission. 85.6% of the newborns admitted had already received antibiotics for various indications at the time of admission before the CSF was tapped. Radiological abnormalities were seen in 16 cases, and eight cases each of meningitis and intracranial hemorrhage were noted.

CSF sample

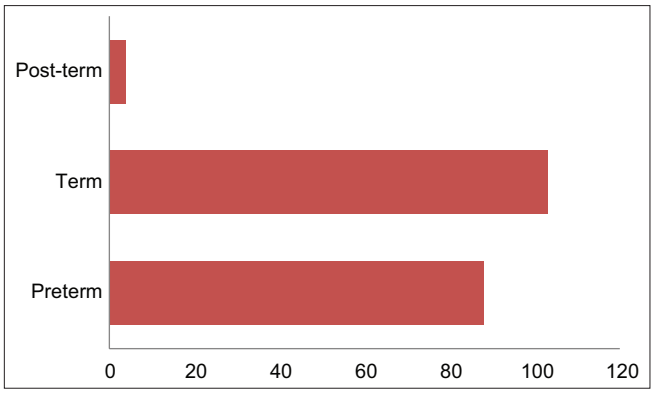
The physical examination, cell count, and biochemical findings of the 213 samples are summarised in Table 1. The mean CSF cell count was 5.48/cu.mm. Neither the Grams stain nor the CSF culture showed any bacteria in any of the cases. *Acinetobacter baumanni* was the most frequently isolated organism in blood culture.

CSF cytologic findings

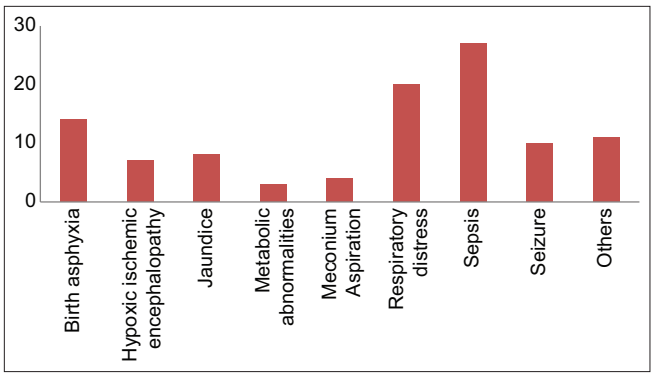
Overall, the most frequent cytologic finding was occasional lymphocytes with or without RBCs (63.9%) [Graph 3 and Figure1]. One sample from a preterm baby showed hemosiderin-laden macrophages along with numerous RBCs, while another sample from another preterm showed arachnoid and choroid plexus cells in a background of many RBCs, respectively.

Correlation between CSF cell count, cytology and clinicoradiological, biochemical and microbiological findings

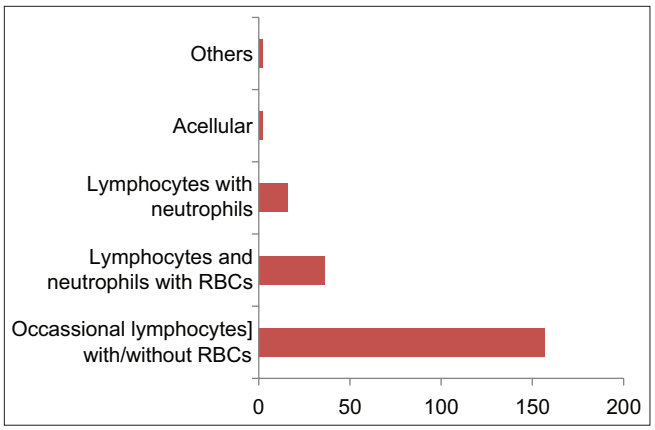
The period of gestation of the neonates ranged between 27 and 42 weeks (mean 35.9 ± 3.6). The birth weight ranged between 640 and 4500 g (mean 2393.3 ± 795.9 g). CSF cell count, as well as CSF neutrophil percentage, did not show a significant correlation with birth weight or period of gestation [Graph 4].



Graph 1: Distribution of cases based on maturity at birth



Graph 2: Distribution of cases based on the clinical diagnosis at the time of admission into the neonatal intensive care unit



Graph 3: Showing cellular composition of the cytocentrifuged cerebrospinal fluid smears. **(***Others include two samples from two preterm babies. One showed hemosiderin-laden macrophages along with numerous RBCs, while the other showed arachnoid and choroid plexus cells in a background of many RBCs***)**

The CSF leucocyte count and protein levels showed a significant correlation with highly sensitive CRP. The CSF cytologic findings showed a significant correlation between the age of neonate and blood neutrophil percentage. ($P = 0.0158$).

Subgroup analysis of various categories

a. *Term versus preterm:* The mean CSF cell counts did not show a significant difference in 'preterm' versus 'term' ($P = 0.8578$),

b. Presence and absence of seizure: The mean CSF cell counts did not show a significant difference in the presence or absence of seizure ($P = 0.1555$)

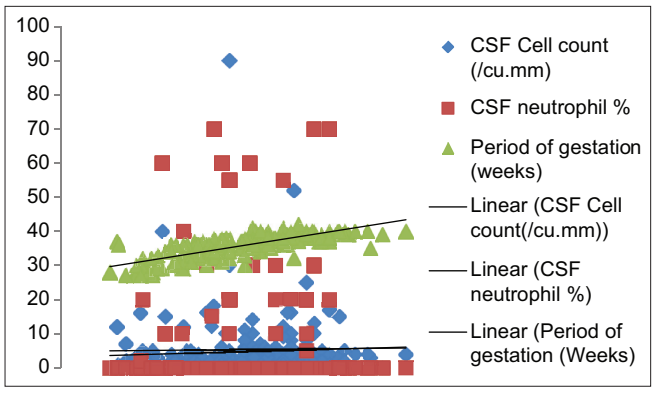
c. Sepsis versus non-sepsis: The mean CSF cell counts did not show a significant difference in sepsis versus non-sepsis. ($P = 0.3230$) [Graph 5].

d. Intracranial hemorrhage: The neonates with intracranial hemorrhage showed a significantly higher frequency of RBCs on cytology. One case, in addition, showed the presence of hemosiderin laden macrophages too, who on, at the time of discharge, showed significant neurological deficits [Table 2 and Figure 2].

e. Meningitis. The cytological patterns seen in cases of confirmed meningitis were significantly different from those of the control group [Table 2 and Figure 3]. Details of the cases of meningitis are discussed in Table 3. Five out of eight cases were preterm, and many of them had risk factors, including a history of maternal chorioamnionitis.

Table 1: Summary of the physical examination, cell count, biochemical and microbiological findings of neonatal cerebrospinal fluid (CSF) findings (*n*=213)

Parameter	Findings	
Physical examination		
Quantity	Mean: 0.5 mL Range: 0.2-1.2 mL	
Colour	Colourless Pale yellow Reddish	25% 69% 06%
Appearance	Clear Hazy	72% 28%
Cell count		
CSF cell count	Mean: 5.48/cu.mm Range: 0-90 cells/cu.mm	
Biochemical evaluation		
CSF glucose	Mean: 66.9 mg/dL Range: 12-337 mg/dL	
CSF protein	Mean: 86.8 mg/dL Range: 30.7-390 mg/dL	
Serum C-reactive protein	Mean: 1.8 mg/dL Range: 0.01->16.7 mg/dL	
Microbiological findings		
Gram stain		
Pus cells±RBCs	Six cases (0-6/oil immersion field)	
Bacteria	Absent in all	
CSF culture		
Bacterial growth	Absent in all	
Blood culture		
Bacterial growth	*Enterococcus fecium* 01 *Acinetobacter baumanni* 03 *Serratia plymuthica* 01 *Klebsiella pneumonia* 01 *Enterobacter cloacae* 01	

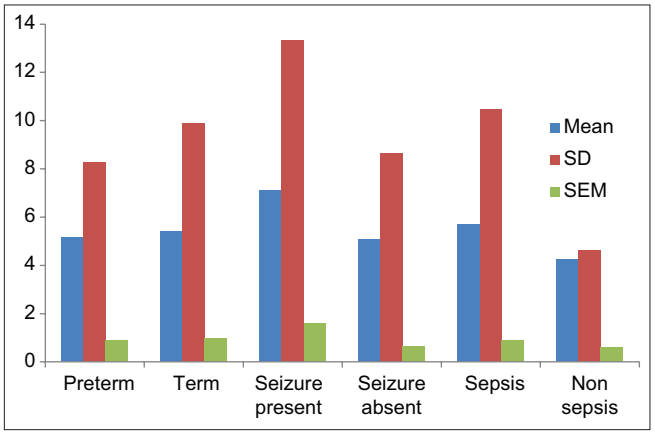


Graph 4: Relationship between cerebrospinal fluid (CSF) cell count, CSF neutrophil percentage, and period of gestation

Discussion

Cytological evaluation of the CSF and assessment of its cellular composition provide valuable first information regarding the underlying pathology.[1] As is highlighted by this study, there are varied CNS and extra-CNS conditions in neonates that necessitate it. There are many ways in which the neonatal CSF analysis is unique compared to that of adults. The BBB between brain parenchyma, cerebral vessel lumen, and epithelial cells by tight junctions plays a significant role in the normal maturation and development of the brain.[2] The neocortex, as studied in rodents, has shown vascularity only after birth.[3] The new hypothesis is that the varied CSF composition at various stages of CNS maturation represents the evolving barrier mechanism during neurogenesis.[3] As is highlighted by this study, babies are admitted for various indications in NICU, with preterm babies constituting a sizeable bulk of cases. Among these, sepsis is the most common, followed by respiratory distress, birth asphyxia, and hypoxic-ischemic encephalopathy.[3] Overall, the incidence of meningitis is maximum in the neonatal period, with greater predilection in preterm neonates resulting in severe neurodevelopmental issues on follow-up.[4,5]All of these require CSF assessment.

The quality of the CSF is crucial during evaluation. In this study, 6% of hemorrhagic samples were seen though the actual cases with intracranial hemorrhage (ICH) constituted only 3.8%. Lumbar puncture, being blinded, is prone to microtrauma resulting in contamination by peripheral blood.[4] The incidence



Graph 5: Subgroup analysis of cerebrospinal fluid (CSF) cell count in various clinical categories (x axis: clinical categories; y-axis shows CSF cell count/cu.mm)

Table 2: The relationship between cytologic and clinicoradiologic findings

Radiologic imaging findings	Parameters	Observations	*P*
Abnormalities on imaging (*n*=16)	Age (in days)	10.25 (2-27)	*P*=0.9849
	M: F	7:9	
	TLC		
	Mean (/cu.mm)	6.7	
	Range (/cu.mm)	0-40	
	SD (/cu.mm)	9.30	
	DLC	Lymphocyte 92.5	
	Mean (%)	40-100	
	(Range in %)	Neutrophil 08.5	
	Mean (%)	0-60	
	(Range in %)		
	Cytology findings	Number of cases	*P*=0.5489
	Lymphocytes only	10	
	Neutrophils, lymphocytes, and RBCs	06	
Intracranial hemorrhage (*n*=8)	Age		*P*=0.8232
	Mean (days)	6.5	
	Range (days)	3-13	
	M: F	6:2	
	TLC		
	Mean (/cu.mm)	6	
	Range (/cu.mm)	2-12	
	SD (/cu.mm)	6.36	
	Cytology	Number of cases	*P*=0.0147
	Lymphocytes only	1	
	Neutrophils, lymphocytes, and RBCs	7	
Meningitis (*n*=8)	Age		*P*=0.0794
	Mean (days)	9.87	
	Range (days)	2-26	
	M: F	7:1	
	TLC		
	Mean (/cu.mm)	27.75	
	Range (/cu.mm)	2-90	
	DLC		
	Mean (%)	Lymphocyte 60.43	
	SD %	37.12	
	Mean (%)	Neutrophil 32.43	
	SD %	26.98	
	Cytology	Number of cases	*P*=0.0147
	Lymphocytes only	01	
	Neutrophils, lymphocytes, and RBCs	07	
No central nervous system abnormalities (*n*=8) (control group used for comparison)	Age (in days)	6.75 (2-21)	
	M: F	5:3	
	TLC		
	Mean (/cu.mm)	6.75	
	Range (/cu.mm)	0-25	
	SD (/cu.mm)	6.11	
	DLC		
	Mean (%)	Lymphocyte	
	(Range in%)	88.75 30-100	
	Mean (%)	Neutrophil 22.25	
	(Range in%)	0-70	
	Cytology	Number of cases	
	Lymphocytes only	06	
	Neutrophils, lymphocytes, and RBCs	02	

of traumatic CSF taps in children ranges from 12% to 26% in studies and can be as high as 47.7% amongst neonates.[5,6] These are affected by the experience of the performer as well as the age and position of the child. Younger neonates tend to have more traumatic punctures due to procedural difficulties.[6] In traumatic taps, the source of WBCs, particularly the polymorphs, is difficult

Table 3: Cytologic details of neonates with meningitis

Term	Clinical diagnosis	CSF cell count (/cu.mm)	CSF neutrophil (%)
Term	Perinatal depression; sepsis	90	55
Preterm	Septic shock with pneumonia	40	60
Term	Seizure; jaundice	10	10
Preterm	Maternal chorioamnionitis	2	0
Term	Methylmalonic aciduria	8	0
Preterm	Klebsiella sepsis	4	0
Preterm	Thigh-staphylococcal abscess	16	2
Preterm	Acinetobacter sepsis	52	0

CSF=Cerebrospinal fluid

to identify.[4] CSF from cases with ICH showed a significantly higher frequency of RBCs on cytology but no difference in cell counts as compared with those having normal imaging findings. Monocytes with phagocytosed RBCs or erythrophages are formed 12–18 hours after ICH. An occasional case in this study showed the presence of erythrophages with a poor neurological outcome. They are a sign of *in vivo* rather than *in vitro* hemorrhage, likely formed and activated by the injury to the choroid plexus and ependymal lining.[7] There could also be differences in the temperature and incubation time in *in vivo* versus *in vitro* settings, which cause them to form only in *in vivo*.[7,8] These erythrophages, after 36 to 48 hours, form hemosiderin deposits and are called siderophages. They persist months after the bleeding.[8,9] In cases of intracranial bleeding, the presence of frank RBCs with erythrophages or siderophages indicates a long-standing IC bleed with a poor neurological outcome and discriminates from a traumatic tap.

The mean leucocyte count in CSF in this study was 5.48/cu.mm, with no significant difference between the term or preterm neonates. Though findings have been found in other studies, this could be due to the smaller sample size in the study, and larger studies are required to confirm the same.[10-12] Another interesting finding in this study is that a seizure history before the procedure did not affect the CSF cell count. Studies with similar findings have proposed that though theoretically, post-ictal CSF pleocytosis can hamper CSF evaluation, practically, it is seen in only 3%–5% of neonates. Further, the degree of pleocytosis in such cases is very mild and about 8/cu.mm. Underlying neurologic disease, concurrent meningitis, and a traumatic tap may be actual contributors to pleocytosis rather than actual postictal effects.[13]

Sepsis was a forthcoming indication for CSF assessment in the present study because of its association with meningitis. But there is conflicting evidence on the nondescript use of lumbar puncture in all cases of sepsis.[14] Lumbar puncture, especially if prolonged, has been found to cause hypoxemia.[15] Also, here, no statistically significant difference was seen in the CSF cell count between sepsis and non-sepsis. So, CSF cytology becomes crucial. This is made further complex by the lack of specific manifestations in neonatal meningitis and may even be sine pleocytosis.[16,17] CSF biomarkers pose a higher cost burden and are confounding due to wide variation in neonates.[16] What compounds the problem, as highlighted here, is the administration of antibiotics for various indications, including a positive septic screen. This is particularly seen in babies referred from peripheral centres. Though *Klebsiella, Enterobacter,* and *Acinetobacter* are common causes of neonatal meningitis, here, none of the cases showed any growth in the CSF culture.[18] Khater *et al.*[19] concluded that the use of antibiotics could lead to CSF-culture-negative meningitis, wherein the use of molecular techniques could prove useful. In this study, the CSF leucocyte count and protein levels showed a significant correlation with highly sensitive CRP. CSF cytology correlated with the blood neutrophil percentage, and a higher incidence of CSF neutrophils was seen in cases of confirmed meningitis. CRP is an acute phase reactant having a homopentameric structure.[20] It is transcriptionally induced in the liver by the CRP gene present at 1q23.2 in response to inflammatory cytokines, IL6. It is increased in various inflammatory conditions like rheumatoid arthritis and the presence of infections. Deviation occurs by 25% in inflammatory conditions and may vary depending on the nature and severity of the underlying disease, as seen in this study.[20] Significantly higher CRP levels are associated with bacterial pathogens.[16] Blood neutrophil percentage is a hallmark of ongoing bacterial infections and sepsis.

So, CSF cytology offers a low-cost alternative in resource-limited settings.

Around 22% prevalence of meningitis is seen in cases of late-onset sepsis, though the incidence of meningitis in neonatal sepsis is 0.3%–3%.[21] In this study also, the preterm neonates and those with a risk factor including chorioamnionitis or abscess have shown coexistence of meningitis. The CSF cytology and cell count, CSF neutrophils, blood neutrophil percentage, and CRP showed to have a good correlation with meningitis in this study, particularly in the appropriate clinical setting. This study throws light upon the common causes of CSF abnormalities in a neonatology unit of a tertiary care centre.

A lot of upcoming research has been done on the usefulness of procalcitonin in neonatal meningitis. Studies have shown that CSF procalcitonin is more associated with meningitis in neonates.[22] It can serve as an add-on to the diagnostic marker.[23] Studies have even found them to be more useful than serum-based assays.[24] However, this pose acts as an expensive affair. This study is, therefore, of importance for the primary care physicians (PCPs) who, with the help of a simple, low-cost test, can provide insight into a likely cause for the newborn illness. The CSF cytology and blood neutrophils percentage can simulate the more expensive CRP levels and procalcitonin, which needs more advanced laboratory investments, serving as a cost-effective alternative. Clinical correlation and the presence of risk factors should direct the PCPs to rule out various treatable conditions.

Despite its limitations, the study has its own merits. Firstly, it shows that the clinical, radiological, and biochemical inputs

are very important in interpreting the CSF abnormality, implying the need for multimodality data integration. Secondly, the study showcases that a cytological assessment has an independent bearing on the diagnosis and follow-up. Thirdly the antibiotic-treated culture-negative cases showed significant abnormalities in CSF cytology which can guide therapy. Next, the significant correlation between blood neutrophil percentage and CRP shows how the CSF mirrors acute changes in meningitis. Lastly, the presence of a good number of RBCs in association with hemosiderin-laden macrophages helps diagnose cases of intracranial hemorrhage in atraumatic cases.

The demerits of this study are that it is a retrospective record-based study. Despite the large sample size, the individual groups had only a few participants per group. So, larger prospective studies on defined cohorts can help understand the complex relationships between CSF abnormalities and other biochemical and microbiological parameters. A longer follow-up period would have been more useful in predicting the impact of CSF cytology on the neurodevelopmental outcomes.

Conclusion

Most studies in the literature on neonatal CSF focus on the role of cell counts only, with limited studies attempting to correlate neonatal CSF cytology with other parameters. Most previous studies have been confined only to meningitis or sepsis alone. Here a broader study has been attempted to cover the role of CSF cytology on the various diagnoses affecting neonates in a NICU. A basic cytologic evaluation of the CSF provides more clinically useful information than mere counts and differentials can provide. It gives a significant insight into an underlying pathology with a low cost and quick method. Accurate cell counts and cytology of neonatal CSF, along with radiological, biochemical, and microbiological workup, can help diagnose and manage neonates in intensive care, especially useful in antibiotic-treated cases.

Key points

1. This study highlights a low-cost alternative in the form of CSF cytology and blood neutrophils percentage for neonatal meningitis.
2. Clinical correlation and the presence of risk factors should direct the primary care physicians to rule out various treatable conditions- clinical, radiological, and biochemical inputs being very important.
3. CSF cytology can guide therapy even in antibiotic-treated culture-negative cases.

Author contribution

Prita Pradhan (PP), Reshmi Mishra (RM), Santosh Panda (SP), Ranjita Panigrahi (RP) were responsible for the planning and conceptualization of the paper. Rajlaxmi Sarangi (RS) contributed the biochemical data while Kumudini Panigrahi (KP) contributed the microbiology data, and Urmila Senapati (US) was responsible for providing the cytology data and cytosmear review. RM and SP provided the clinical inputs. PP did the statistical analysis. PP and RM contributed to the microphotographs. The manuscript was drafted primarily by PP, but all authors have read and approved the manuscript.

Acknowledgement

The authors would like to thank Mr Vara Prasad SVN, Medical Records Officer at Kalinga Institute of Medical Sciences, for providing them with access to the medical records.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Rahimi J, Woehrer A. Overview of cerebrospinal fluid cytology. Handb Clin Neurol 2017;145:563-571. 4.
2. Saunders NR, Dreifuss J-J, Dziegielewska KM, Johansson PA, Habgood MD, Møllgård K, *et al.* The rights and wrongs of blood-brain barrier permeability studies: A walk through 100 years of history. Front Neurosci 2014;8:404.
3. Celik IH, Demirel G, Canpolat FE, Dilmen U. A common problem for neonatal intensive care units: Late preterm infants, a prospective study with term controls in a large perinatal center. J Matern Fetal Neonatal Med 2013;26:459-62.
4. Krishnamurthy V, Nabil N, Reddy SM, Doreswamy SM. Dilemma in diagnosis of pyogenic meningitis in cerebrospinal fluid contaminated with blood: Does leucocyte esterase test help? J Cytol 2019;36:44.
5. Glatstein MM, Zucker-Toledano M, Arik A, Scolnik D, Oren A, Reif S. Incidence of traumatic lumbar puncture: Experience of a large, tertiary care pediatric hospital. Clin Pediatr (Phila) 2011;50:1005-9.
6. Matettore A, Kollmann TR. Traumatic neonatal lumbar punctures: Experience at a large pediatric tertiary care center in Canada. Am J Perinatol 2018;35:764-8.
7. Dersch R, Benkler D, Robinson T, Baumgartner A, Rauer S, Stich O. Erythrophages do not develop when lumbar CSF and blood samples are mixed in vitro. Fluids Barriers CNS 2018;15:31.
8. Schwenkenbecher P, Janssen T, Wurster U, Konen FF, Neyazi A, Ahlbrecht J, *et al.* The influence of blood contamination on cerebrospinal fluid diagnostics. Front Neurol 2019;10:584. doi: 10.3389/fneur. 2019.00584.
9. Nagy K, Skagervik I, Tumani H, Petzold A, Wick M, Kühn H-J, *et al.* Cerebrospinal fluid analyses for the diagnosis of subarachnoid haemorrhage and experience from a Swedish study. What method is preferable when diagnosing a subarachnoid haemorrhage? Clin Chem Lab Med 2013;51:2073-86.
10. Majumdar A, Jana A, Jana A, Biswas S, Bhatacharyya S, Bannerjee S. Importance of normal values of CSF parameters in term versus preterm neonates. J Clin Neonatol 2013;2:166-8.

11. Martín-Ancel A, García-Alix A, Salas S, Del Castillo F, Cabañas F, Quero J. Cerebrospinal fluid leucocyte counts in healthy neonates. Arch Dis Child Fetal Neonatal Ed 2006;91:F357-8.
12. Srinivas L, Shah S, Padula MA, Abbasi S, McGowan KL, Harris MC. Cerebrospinal fluid reference ranges in term and preterm infants in the neonatal intensive care unit. J Pediatr 2012;161:729-34.
13. Wong M, Schlaggar BL, Landt M. Postictal cerebrospinal fluid abnormalities in children. J Pediatr 2001;138:373-7.
14. Ray B, Mangalore J, Harikumar C, Tuladhar A. Is lumbar puncture necessary for evaluation of early neonatal sepsis? Arch Dis Child 2006;91:1033-5.
15. Duru CO, Ederiane OE, Allen-Ameri D, Ibrahim ZF, Pondei K, Aliyu I. Does hypoxemia occur before, during or after lumbar puncture procedure in ill newborns? Our experience in a tertiary health center. J Clin Neonatol 2017;6:173-8.
16. de Blauw D, Bruning A, Vijn LJ, Wildenbeest JG, Wolthers KC, Biezeveld MH, *et al.* Blood and cerebrospinal fluid characteristics in neonates with a suspected central nervous system infection. Medicine (Baltimore) 2019;98:e16079.
17. Yun KW, Choi EH, Cheon DS, Lee J, Choi CW, Hwang H, *et al.* Enteroviral meningitis without pleocytosis in children. Arch Dis Child 2012;97:874-78.
18. Boskabadi H, Heidari E, Zakerihamidi M. Etiology, clinical findings and laboratory parameters in neonates with acute bacterial meningitis. Iran J Microbiol 2020;12:89-97.
19. Khater WS, Elabd SH. Identification of common bacterial pathogens causing meningitis in culture-negative cerebrospinal fluid samples using real-time polymerase chain reaction. Int J Microbiol 2016;2016:4197187. doi: 10.1155/2016/4197187.
20. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol 2018;9:754.
21. Kaul V, Harish R, Ganjoo S, Mahajan B, Raina SK, Koul D. Importance of obtaining lumbar puncture in neonates with late onset septicemia a hospital based observational study from north-west India. J ClinNeonatol 2013;2:83-7.
22. Dutta S, Sachdeva N, Pal A, Ray P. Cerebrospinal fluid and plasma procalcitonin for the diagnosis of neonatal bacterial meningitis. J Paediatr Child Health 2022;58:1425-30.
23. Nagaraj M, Bandiya P, Jagannatha B, Shivanna N, Benakappa N, Bandyopadhyay T. Diagnostic utility of cerebrospinal fluid procalcitonin in neonatal meningitis. J Trop Pediatr 2022;68:fmac043.
24. Rajial T, Batra P, Harit D, Singh NP. Utility of cerebrospinal fluid and serum procalcitonin for the diagnosis of neonatal meningitis. Am J Perinatol 2022;39:373-8.