Late-Onset Sepsis in Preterm Neonates is Associated with Higher Risks of Cerebellar Hemorrhage and Lower Motor Scores at 3 Years of Age

Mais Kartam[1], Alia Embaireeg[2], Shahad Albalool[2], Awrad Almesafer[3], Majeda Hammoud[4], Monif Al-Hathal[4] and Mariam Ayed[5*]

[1]Pediatric Department, Kuwait Institute for Medical Specializations, Ministry of Health, Kuwait
[2]Pediatric Department, Farwaniya Hospital, Subah An Nasser, Kuwait
[3]Pediatric Department, Amiri Hospital, Kuwait City, Kuwait
[4]Neonatal Department, Maternity Hospital, Sabah, Kuwait
[5]Neonatal Department, Farwaniya Hospital, Sabah An Nasser, Kuwait

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[*]***Corresponding author*:** mariam.ayed@hsc.edu.kw

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Abstract

Background: Sepsis is associated with adverse neonatal outcomes, including diffuse white matter injury (WMI) which may predispose to developmental delay.

Objective: To evaluate the impact of late-onset sepsis (LOS) in preterm infants on brain injury and neurodevelopmental outcomes at 36 months corrected age (CA).

Design: Retrospective cohort study.

Setting: Neonatal Sepsis Registry at Neonatal Department, Al-Sabah Maternity hospital, Kuwait.

Participants: A total of 203 neonates (gestational age (GA) between 24-32 weeks) were admitted between January 2017 and December 2017. Neonates were stratified into no sepsis, into early-onset sepsis (first onset of sepsis ≤72 hours postnatally), and LOS (>72 hours postnatally)

Main outcome: Brain injury and neurodevelopmental outcomes at 36 months CA were evaluated using Miller score and Bayley-III scales of infant development, respectively.

Results: Sixteen neonates had early-onset sepsis with *Klebsiella pneumonia* and group-B streptococcus, and 93 developed LOS with *K. pneumonia* and gram-positive cocci in clusters. Intraventricular hemorrhage (n=68) and WMI (n=42) showed no group-wise differences. Higher cerebellar hemorrhage risk (adjusted odds ratio=4.6 (1.3-18.6); p=0.03) and lower motor composite scores (adjusted β=-9.5 (-16.4 to -2.7); p=0.007) were observed with LOS.

Conclusions: LOS in preterm neonates is a significant risk factor predisposing to cerebellar hemorrhage and lower motor scores by three years of age.

Keywords: Neonate, newborn, sepsis, infection, brain injury, hemorrhage, neurodevelopmental

INTRODUCTION

Over the past decade, advanced neonatal intensive care has remarkably improved the survival rates of premature infants; however, prematurity and infectious diseases continue to be notable reasons for infant morbidity and mortality.[1,2] Neurodevelopmental outcomes in the surviving neonates have been disappointing, especially those with very low birth weight (VLBW) less than 1500 grams and preterm infants. Sepsis is an established contributor to poor neurodevelopmental outcomes in preterm neonates, with more susceptibility to developing late-onset sepsis (LOS)[3,] which has a reported prevalence of 20-50 %, and higher rates are associated with lower gestational age and birth weight.[4,5] This increased susceptibility to NICU-induced LOS may be attributed to a prolonged hospital stay, the need for invasive procedures like intubation and central line insertions, delayed enteral feed, and early exposure to broad-spectrum antibiotics.[1,6,7]

A majority (85%) of the nosocomial infections contracted in the NICU and subsequent LOS are caused by Gram-positive bacteria, mainly coagulase-negative Staphylococci (CONS) bacteria (55%).[8] However, Gram-negative infections also result in significant neonatal morbidity and mortality. Further, LOS may or may not cause meningitis irrespective of the mode of infection, i.e., via the hematogenous route (through the choroid plexus) or direct contamination of any open wound, central line, fetal scalp monitor, or neural tube defect.[9]

Brain maturation and neurological development occur primarily during the last trimester of pregnancy, continuing up to the early postnatal years.[10] In a preterm neonate, the immature brain, especially the oligodendrocytes lineage, is extremely vulnerable to a hypoxic-ischemic insult, hemorrhage, or a systemic inflammatory reaction ensuing against infections irrespective of the origin - maternofetal transmission or postnatal during the NICU stay. Several mechanisms, like an endotoxin-induced cytokine storm, disturbed auto-regulation, excitotoxicity, and inadequate perfusion, have been proposed to describe the pathogenesis of sepsis that damages the immature brain. An association between intraventricular hemorrhage (IVH) or periventricular white-matter lesions and the release of IL-6 (a pro-inflammatory cytokine) resulting from intrauterine infection has been reported, substantiating the hypothesis of a similar course postnatally during LOS.[11] Further, it is known that preterm infants with LOS, even without meningitis, have a considerable risk of poorer neurodevelopmental outcomes.[12] Similarly, gastrointestinal infections like necrotizing enterocolitis (NEC) may trigger systemic inflammatory response resulting in significant neuronal injury[13,14] with consequent neurodevelopmental implications visible at 18-22 months follow-up.[7,15,16]

Neuroimaging is an important diagnostic tool in ensuring competent NICU care and determining prognosis in case of neonatal comorbidities.[17] Magnetic resonance imaging (MRI)

of a preterm neonate's brain, which is extremely vulnerable to IVH and white matter injury (WMI), to localize the lesion may give an important insight into the possible motor and cognitive deficits that may manifest with age.[18]

Multiple clinical studies have shown an increased risk of WMI and IVH with sepsis[19-21]; however, limited studies have analyzed the risk of brain injury with the onset of sepsis. The objective of the study is to evaluate the impact of late neonatal sepsis on brain injury using MRI and its consequences on neurodevelopmental outcomes by three years of age.

METHODS

Study population

A retrospective analysis of the medical records obtained from the Neonatal Sepsis Registry at Neonatal Department, Al-Sabah Maternity Hospital, Kuwait, was done. All infants with a gestational age < 32 weeks, excluding those with congenital anomalies or syndromes, admitted to a Level-III NICU at the Al-Sabah Maternity Hospital, Kuwait, between January 2017 through December 2017 were included in the study cohort. The study was approved by the Ethics Committee, Ministry of Health, Kuwait (2016/1420).

Defining the variables

The baseline characteristics for all patients were collected from the medical charts. Neonatal sepsis confirmed using a positive culture was defined as any blood and/or cerebrospinal fluid sample positive for bacterial growth. Blood culture was considered a contaminant for Gram-positive cocci in a cluster if the second culture drawn 30-minutes apart was negative. Early-onset sepsis (EOS) was described as any positive blood culture occurring ≤ 72 hours after birth, and LOS was an infection contracted after this period.[22] We considered sepsis as severe if associated with hemodynamic instability and disseminated intravascular coagulopathy (DIC).

Systemic hypotension was characterized by systolic or diastolic or mean blood pressure below the 3rd centile for age needing inotropic support. NEC was categorized using Bell's criteria (stage ≥ 2).[23] A patent ductus arteriosus (PDA) was diagnosed based on echocardiographic evidence of a hemodynamically significant PDA requiring pharmacological or surgical treatment. Bronchopulmonary dysplasia (BPD) or chronic lung disease (CLD) was designated when supplemental oxygen was required by the 28th day postnatally or week 36 postmenstrual age (PMA).[24] Severe retinopathy of prematurity (ROP) grades 3 and 4 were defined using the International Classification of Retinopathy of Prematurity.[25]

Outcomes

A brain MRI (1.5 or 3 Tesla) was performed in 181 neonates after swaddling, feeding, or sedation with 25-50mg/kg chloral hydrate. Besides the standard T1 and T2 weighted images, Fluid Attenuated Inversion Recovery (FLAIR), apparent diffusion coefficient (CDC), and diffusion-weighted images (DWI) sequences were obtained and interpreted by an experienced neuroradiologist. The Papile system was used to grade IVH.[26] WMI was defined according to Miller's scoring system into mild WMI (< 3 areas of abnormal T1 signal intensity), moderate (> 3 areas of abnormal T1 signal intensity and < 5% hemispheric involvement), and severe (> 5% of the hemisphere involved).[18] The presence of cerebellar hemorrhage was also recorded.

Neurological development by 36 months corrected age (CA) was evaluated using the Bayley Scales of Infant and Toddler Development-III (BSID-III) - cognitive, language, and motor composite scores.[27] A 'moderate' developmental delay was designated to a worst composite score of 70-84 in ≥ 1 of the 3 domains. Whereas, a score of < 70 for any of the 3 domains or when unable to assign a score owing to severe mental deficiency or cerebral palsy (appraised using the Gross Motor Function Classification System (GMFCS)) was termed as

'severe' delay.[28] The GMFCS evaluates the gross motor function of children and youth with cerebral palsy (CP) considering their ability to initiate movements for basic tasks like sitting and ambulation - walking or wheeled mobility.

Statistical analysis

Descriptive statistics were calculated for all data - median, interquartile (IQR) for continuous variables, and frequency (%) for categorical variables. Fisher's exact test for qualitative and Kruskal–Wallis test for quantitative data were used to compare baseline clinical and imaging characteristics of the 3 study groups. Correlation between sepsis and other clinical variables relevant to brain injury and neurodevelopmental outcomes was computed using univariate linear regression. Similarly, multivariate analysis was done to compare the outcomes at 36-month for early and late-onset sepsis. The association between infections and their 36-month outcomes was calculated using both unadjusted and adjusted β coefficients. All analyses were performed using Stata/IC 14.2 (Stata Corp, College Station, Texas) and a p-value <0.05 indicated statistical significance.

RESULTS

Two-hundred-three neonates born at the gestational age of 24-32 weeks and admitted to the NICU were included in the study, of which 93 (45.8%) remained free from any sepsis, 16 (7.9%) had EOS, and 94 (46.3%) developed a LOS (Table 1). Forty-one (44.6%) of the 94 newborns with LOS had histological evidence of chorioamnionitis. Also, the LOS group had a median birth weight of 0.84 kg (IQR = 0.69-0.97 kg; p <0.001) versus > 1 kg for other groups. Likewise, the LOS group had similar trends for gestational age ($p < 0.001$), length ($p < 0.001$), and head circumference ($p < 0.001$). Neonates in this group were more likely to develop PDA, NEC stage ≥ 2, CLD, and ROP ($p < 0.001$). *Klebsiella pneumonia* (31.2%) was the commonest organism

causing EOS, followed by *Streptococcus agalactia* (25%) and *Escherichia coli* (18.7%). Similarly, among the LOS group, *Klebsiella pneumonia* (57.4%) had the highest prevalence, followed by CONS, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and Serratia (27.6%).

Table 1 Clinical characteristics and MRI findings of the neonates compared by the onset of infection

Variables	**No sepsis(n=93)**	**Early-onset sepsis (n= 16)**	**Late-onset sepsis (n= 94)**	**p-value**
Antenatal steroid (%)	83 (90.2%)	12 (80%)	85 (90.4%)	0.458
Histological chorioamnionitis (n=74) (%)	24 (26.7%)	9 (56.3%)	41 (44.6%)	0.01
Gestational age, (weeks) Median (IQR)	29.5 (28.1-31.4)	27 (25.8-28.7)	25.9 (25-27.7)	<0.001
Birth weight (grams) Median (IQR)	1190 (1020-1380)	1045 (832-1335)	840 (695-966)	<0.001
Head Circumference at birth (cm) Median (IQR)	27 (25.5-28)	24 (23.5-26)	24 (22.5-26)	<0.001
Length at birth (cm) Median (IQR)	38 (36-40)	35 (33-38.5)	34 (31.5-37)	<0.001

Male (%)	48 (51.6%)	8 (50%)	52 (55.3%)	0.369
10 min Apgar score Median (IQR)	8 (7-9)	8 (7-9)	8 (7-8)	0.09
PDA (%)	25 (27.2%)	10 (62.5%)	60 (63.8%)	<0.001
NEC stage ≥2(%)	9 (9.8%)	4 (25%)	32 (34%)	<0.001
ROP (%)	20 (21.5%)	10 (62.5%)	63 (67%)	<0.001
CLD (%)	5 (5.4%)	1 (6.3%)	31 (32%)	<0.001
Hypotension (%)	17 (18.3%)	5 (31.2%)	58 (61.7%)	<0.001
Severe sepsis (%)	0	4 (25%)	52 (55.3%)	<0.001
Meningitis (%)	0	0	2 (2.1%)	0.574
White matter injury, (%) n=181	8 (9%)	2 (13.3%)	8 (10.5%)	
Mild	7(7.9%)	3 (20%)	5(6.5%)	0.586
Moderate	4 (4.5%)	1 (6.7%)	4 (5.3%)	
Severe				

IVH (%)				
Grade I	3 (3.7%)	4 (26.7%)	5 (6.5%)	
Grade II	23 (25.8%)	4 (26.7%)	22 (28.6%)	0.07
Grade III	2 (2.2%)	0	5 (6.5%)	
IVHgrade III-IV (%)	25 (28.1%)	4 (26.7%)	27 (35.1%)	0.595
Cerebellar hemorrhage (%)	5 (5.6%)	3 (20%)	23 (28.7%)	<0.001
Organisms				
Klebsiella pneumonia (%)	0	5 (31.2%)	54 (57.4%)	0.01
Escherichia coli (%)	0	3 (18.7%)	6 (6.4%)	0.452
Enterococcus faecalis (%)	0	2 (12.5%)	4 (4.2%)	0.310
Candida (%)	0	0	3 (3.2%)	0.06
Streptococcus agalactiae (%)	0	4 (25%)	1 (1%)	0.001
Others (%)	0	1 (6.2%)	26 (27.6%)	0.002

Others include coagulase-negative *Staphylococcus*, *Staphylococcus epidermidis*, and Methicillin-resistant *Staphylococcus aureus*, Serratia

A total of 181/203 neonates underwent MRI brain at a median gestational age of 34 weeks (IQR = 33-36 weeks). Out of the remaining 22/208, 18 (8.9%) neonates did not survive, while the parents of the other 4 did not consent for an MRI. Among the 18 who expired, 5 had no sepsis, 10 had LOS from *Klebsiella* infection, 2 had *E. coli* infection and 1 developed LOS from Enterococcus faecalis infection. WMI and IVH were observed in 42 (23.3%) and 68 (37.6%) infants out of the 203, respectively, with no group-wise differences in the risk of WMI and IVH severity ($p > 0.05$). Also, cerebellar hemorrhage on MRI was noted in 31 (17.1%) neonates. Strikingly, a higher proportion of neonates with LOS developed cerebellar hemorrhage compared to those with EOS (28.7% vs. 20%) and those without sepsis (28.7% vs. 5.6%) ($p < 0.001$) (Table 1).

Adjusting for birth weight, gestational age, severe sepsis, and NEC, a significant association was seen between LOS and a higher risk of cerebellar hemorrhage (adjusted odds ratio= 4.6; 95%CI: 1.3 to 18.6). The risk of cerebellar hemorrhage increased after infection with *Klebsiella pneumonia* (58%) and *Enterococcus faecalis* (22.5%) (Table 2).

Table 2 Risk of cerebellar hemorrhage between organisms.

Organism	**No cerebellar hemorrhage** **N= 150**	**Cerebellar hemorrhage** **N= 31**	**p-value**
Klebsiella Pneumonia (%)	43 (28.7%)	18 (58%)	0.003
Escherichia coli (%)	13 (8.7%)	1 (3.2%)	0.471
Enterococcus faecalis	10 (6.6%)	7 (22.5%)	0.012

(%)			
Candida (%)	6 (4%)	4 (12.9%)	0.069
Streptococcus agalactiae (%)	9 (6%)	5 (16.1%)	0.067
Others (%)	29 (19.3%)	11 (35.5%)	0.058

Others include coagulase-negative *Staphylococcus*, *Staphylococcus epidermidis*, and Methicillin-resistant *Staphylococcus aureus*, Serratia)

Neurodevelopmental outcomes were evaluated for 168 infants using the BSID-III scoring by 36 months CA (median = 34 months, IQR = 33-36 months). Between discharge and 34-month assessment, 2 infants died, 8 were lost during follow-up, while 3 had a severe impairment and were unable to complete the test. The univariate analysis revealed a significant association between LOS and lower Bayley-III composite scores for all 3 domains - motor ($p < 0.001$), cognitive ($p = 0.027$), and language ($p = 0.044$). Also, LOS was associated with a higher risk of cerebral palsy (28%, $p = 0.003$) (Table 3). Figure 1 compares the groups based on their BSID-III scale composite scores. However, the multivariate regression analysis (adjusting for gestational age, birth weight, cerebellar hemorrhage, and white matter injury) revealed that the LOS group was associated with significantly lower motor composite scores (β coefficient =-9.5; 95%CI: -16.4 to -2.7), and not cognitive and language composite scores ($p>0.05$) (Table 4).

Table 3 Bayley-III neurodevelopmental outcome at 36 months corrected age

Assessment	**No sepsis** **N= 83**	**Early-onset sepsis** **N= 10**	**Late-onset sepsis** **N= 75**	**p-value**
Bayley-III motor composite score Median (IQR)	107 (100-115)	107 (97-110)	97 (82-107)	<0.001
Bayley-III cognitive composite score Median (IQR)	103 (100-110)	105 (100-105)	100 (90-105)	0.027
Bayley-III language composite score	110.5 (103-118)	112 (106-115)	106 (94-115)	0.044
Cerebral palsy (%)	8 (9.6%)	0	21 (28%)	0.003

Table 4 Unadjusted and adjusted β coefficient value for Bayley-III scores at 36 months corrected age in early and late-onset sepsis compared to no sepsis

Assessment	**Early-onset sepsis**	**Late-onset sepsis**
Bayley-III motor composite score	*-4.1 (-14.4 to 6.2);0.435	*-6.7 (-11.6 to -1.7);0.008
	**-0.1 (-7.7 to 7.5);0.938	**-9.5 (-16.4 to -2.7)'0.007
Bayley-III cognitive composite score	*0.83 (-5.1 to 6.8);0.784	*-4.7 (-9.2 to -0.1);0.046
	** 2 (-5 to 9.1);0.569	**-4.7 (-11.2 to 1.7);0.146
Bayley-III language composite score	*-4.1 (-14.8 to 6.5);0.443	*-5.9 (-11.5 to -0.32)'0.038
	**3.9 (-7.8 to 15.6);0.507	** -0.82 (-8.3 to 6.6); 0.829

*unadjusted, ** adjusted β (95% CI); *P* value

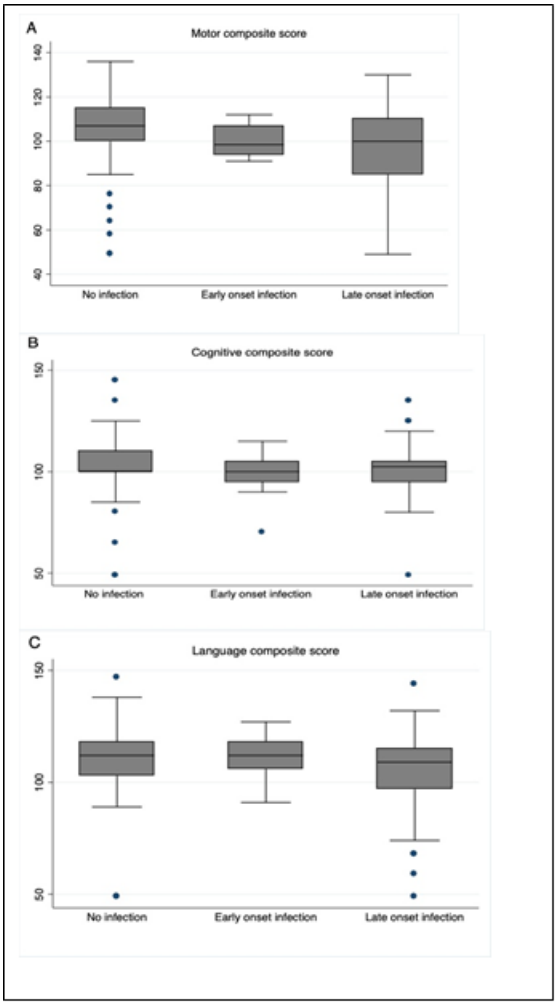

Figure 1 Bayley-III neurodevelopmental outcome at 36 months corrected age. The middle line and box reflect the median and IQR, respectively, with the 1.5 IQR reflected by the whiskers and black circles as any outliers.

DISCUSSION

This study is a unique analysis of neonatal LOS and its influence on brain injury and neurodevelopmental outcomes by 3 years of age. Neonates with LOS had comparatively lower gestational age, birth weight, length, and head circumference. Similarly, cerebellar hemorrhage was highly prevalent with a history of LOS, while the incidence of WMI and IVH was similar

for all neonates irrespective of sepsis history. These results also reaffirm the concept that LOS negatively affects neurodevelopmental outcomes, particularly motor, as evident from the lower BSID-III motor composite scores in the LOS group, which was also susceptible to developing cerebral palsy.

Sepsis may lead to BPD, requiring steroid therapy to facilitate extubation in these patients and mitigate other complications of a preterm birth like adrenal insufficiency and arterial hypotension.[29-31] A series of studies evaluated the incidence of both EOS (1.5 per 1000 live births) and LOS (11.63 per 1000 live births) in the Gulf region, highlighting the disease burden because of LOS contracted in the NICU.[32,33] Furthermore, besides the immediate impact of sepsis and the subsequent systemic inflammatory response, our results establish a significant association of severe sepsis and complications like CLD, PDA, NEC, hypotension, and ROP through univariate logistic regression analysis. These complications adversely affect the developing brain, as demonstrated by Stoll *et al*. (2002), who found that infants with sepsis, or sepsis with NEC, or meningitis were significantly predisposed to poorer neurodevelopmental outcomes evident by 18-22 months.[11,13-15] Likewise, another study described that neonatal sepsis with NEC is associated with increased neurodevelopmental impairment in low birth-weight survivors manifesting as cerebral palsy.[16] Several human and animal studies have verified the presence of bacterial exotoxins-induced cytokine-release from microglia and astrocytes, resulting in a systemic inflammatory response syndrome, which further increases the blood-brain barrier permeability causing neuronal damage and apoptosis.[34-37] The consequent arterial hypotension and the lability of blood pressure during sepsis, coupled with coagulopathy, lead to cerebral ischemia-reperfusion injury impairing neuronal oxygenation and cerebral autoregulation.[11] These

events, supplemented by the biological prematurity of the nervous tissue render it vulnerable to injury, presenting as neurocognitive or neuromotor developmental deficits.[38,39]

Some reports consider CONS bacteria as the commonest organisms causing LOS[12], however, Gram-negative organisms *(K. pneumonia* and *E. coli*) were the main causative organisms for LOS in our study. Also, infants with exceptionally lower gestational age were at a higher risk of cerebellar hemorrhage. Mortality and morbidity caused by Gram-negative bacteria are significantly higher, evident as substantial motor deficits in preterm babies by 3 years of age.[12] Gram-negative bacteria camouflage and conceal the cell antigen by an additional capsule or slime layer covering the cell membrane, which protects it against the host's systemic inflammatory response and prevents early identification. Furthermore, the effect of neonatal sepsis on platelets is organism-specific, and gram-negative bacteria elicit thrombocytopenia and coagulopathy by strongly attacking the platelets.[40] Hence, the higher risk of cerebellar hemorrhage with Gram-negative infection. Barring the effect of location and extent of hemorrhage, 43-75% of infants who develop cerebellar hemorrhage experience serious delays in language, motor, cognitive, and behavioral development.[41]

This study also corroborates the importance of early quantitative MRI done in preterm neonates, more so for neonatal sepsis. Cayam-Rand *et al*. (2019) recommended the use of MRI coupled with clinical factors to detect and localize WMI and predict developmental outcomes by preschool age.[42] Although we did not localize WMI, a significant association of cerebellar hemorrhage on MRI with neurodevelopmental outcomes, particularly motor, can be deduced from our results. These results also highlight the importance of using advanced serial MRI in the NICU to delineate the role of prematurity-induced cerebellar hemorrhage and topographically correlate with the developmental outcomes assessment.

We also identified a strong association between NICU-contracted LOS and developmental deficits due to the resulting brain injury. The processes of cellular migration, proliferation, and arborization essential for the fetus' neurological development occur during the 3rd trimester as a highly dynamic progression. Hence, any insult to the developing brain, be it preterm birth, intrauterine infection, or the systemic inflammatory response to LOS will have neurological consequences, as shown in this study.

The study has some limitations. The retrospective nature of the study may cause a recall bias in data collection; however, the authors managed to retrieve almost all the relevant information. Second, the study sample was small and single-centric, limiting the generalizability of the results. However, our work showed statistical significance enough to prove the association of LOS with cerebellar hemorrhage in premature infants. Future research should cover a larger proportion of the population to identify other confounding factors influencing neurodevelopmental outcomes.

CONCLUSIONS

Late-onset sepsis in a premature newborn leads to a higher probability of cerebellar hemorrhage and other neonatal comorbidities, resulting in poorer neurodevelopmental outcomes. The neurological prognosis further deteriorates with infection with Gram-negative bacteria (particularly *Klebsiella pneumonia* and *Escherichia coli*). This study provides a greater understanding of the risk factors linked with poor neurodevelopmental outcomes that can either be averted or managed using preventive and therapeutic strategies.

DECLARATIONS

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Author Contribution: MK, AE, and MA conceptualized and planned the study design, planned data collection, oversaw data collection, conducted literature review, performed the text mining analysis, drafted and revised the final version of the manuscript. MA also performed the statistical analysis. MH and MAL contributed to the writing and reviewing of the manuscript. All other co-authors contributed to data collection and oversaw the manuscript.

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