Original Article

Comparison of the normal CSF values in premature neonates with term babies

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Abstract

Background: Premature infants are at more risk for meningitis, and lumbar puncture is therefore a necessary part of the sepsis workup. The normal cerebrospinal fluid markers of premature infants have not been well established. This study was conducted to define the normal values of cerebrospinal fluid in these infants. **Materials and Methods:** In this cross-sectional research, we compared the CSF analysis of premature and term neonates admitted to Mahdieh and Mofid children's hospitals from 2014 to 2017. **Results:** Of 248 neonates, 44.4% were females and 55.6% were males. Fifty-five percent were term and 45% were preterm. The mean birth weight and gestational age of term and preterm babies were 3409 ± 65 and 1373 ±376 gram (P value<0.001) and 38.46 and 31.66 weeks respectively (P value<0.001). The mean age of preterm and term patients at the time of lumbar puncture was 25.96 and 9.66 days respectively (P value<0.001). Overall, analysis of the CSF, protein, glucose, WBC, PMN, and RBC revealed the normal values of 92.7%, 79.4%, 89.9%, 91.5%, and 35.5% respectively. By comparing CSF parameters of term and premature babies, it was exhibited that protein was the only CSF parameter that was significantly different between two given groups (10.23mg/dl vs. 71.47 mg/dl, P-value<0.001). The percentages of abnormal CSF parameters in the two groups was not significantly different.**Conclusion:** Our study revealed that the CSF markers of term and preterm neonates are similar; the exception was the protein level so that it was significantly high even in the preterm neonates without meningitis or intraventricular hemorrhage.

Keywords: Premature Infant, Lumbar puncture, Meningitis

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Introduction

Bacterial meningitis is a serious infection that is more common in neonatal periods than the other age groups and causes significant morbidity and mortality [1-3]. Premature infants are at more risk for sepsis and meningitis [4, 5]. Twenty-one to 25% of premature infants and 50% of infants weighing less than 750 grams have one or more episodes of culture-positive sepsis in the first 3 days of life [4, 5]. Five percent of very low birth weight infants who are evaluated for sepsis have meningitis and lumbar puncture (LP) is an indispensable part of the sepsis work-up in these premature infants [1]. Even though values for cerebrospinal fluid (CSF) cell counts, protein concentration, and glucose concentration are well recognized in children and full-term infants, CSF values in premature infants have not been well established. Some few studies are available that assessed CSF values in low birth weight infants in a small number of subjects [6, 7]. Defining the CSF values in premature infants is difficult because of factors such as traumatic LPs, intracranial hemorrhage, and antibiotic use. CSF culture is the gold standard

method for diagnosing bacterial meningitis. But, in neonatal intensive care units (NICU), oftentimes antibiotics are initiated for infants before performing LP. Antibiotic initiation before LP affects the results of cultures and forces clinicians to diagnose meningitis indirectly, from the interpretation of other CSF values [2, 8]. Performing LP as a part of the diagnostic evaluation of suspected sepsis in premature infants is controversial [9]. It is thought that meningitis is under-diagnosed among low birth weight infants and a recent study suggested performing an LP as part of the approaching suspected sepsis in premature babies [8].

Previous studies of CSF values in infants in NICUs were based on retrospective analyses of information from databases, with significant amounts of missing information [6, 10].

Although there is no data indicating the precise incidence of meningitis in the Iranian neonatal population especially in preterm infants, but there are some reports on neonatal meningitis Iran. In a study conducted by Ghabouli-Shahroodi and colleagues, based on real-time polymerase chain reaction (RT-PCR) findings, the incidence of meningitis caused by enterovirus infection was reported 30%. In this study, the authors also showed normal CSF and PMN dominancy is common in neonates and children with enterovirus meningitis [11]. In a recent report by Pormohammad and colleague, it was revealed that of 119 patients, 43 cases had neonatal meningitis, and cerebrospinal fluid WBC, protein, PMN leukocytes, and serum C-reactive protein [12]. Since the diagnosis of bacterial meningitis in neonates is critically important, and few studies have been done to determine the normal ranges of CSF values in low birth weight infants, this descriptive cross-sectional study was conducted to define the normal values of CSF in these infants.

Methods

In this research, we had a cross-sectional non-randomized simple sampling method on the medical records of all low birth weight premature infants (<2000 g) who were admitted to our neonatal intensive care unit (NICU)at Mahdieh and Mofid children's hospitals in Tehran/Iran, between years 2014 and 2017.

All low birth weight premature infants who underwent lumbar puncture (LP) for the evaluation of sepsis were considered for inclusion.

Inclusion criteria. 1. Non-traumatic LP (RBC = 0 / mm3) or traumatized with a maximum of 500 RBC/mm3.2.Clinical and Para clinical information available

Exclusion criteria. 1. Major congenital abnormalities 2.Evidence of intraventricular hemorrhage(IVH) by ultrasound.

As the control group, we evaluate the CSF markers of term neonates (gestational age more than 37 weeks) and compare those markers between two groups.

If patients had multiple LPs during their neonatal intensive care unit admission, the first LP was considered for analysis and later LPs were excluded.

Prematurity was determined in our study with a gestational age of fewer than 37 weeks. Bacteremia was considered as a positive blood culture during the time that the infant was undertaken LP. Bacterial meningitis was determined by the isolation of a bacterial pathogen from CSF. Because sterilization of CSF may occur after antibiotic administration, if antibiotic administration was initiated before LP, bacterial meningitis was detected by the presence of bacteria in the gram stain and high CSF protein which in our analysis: CSF protein above 150 and 170 mg/dl was considered abnormal in terms and preterm neonates respectively. CSF WBC higher than 32/mm3 was considered abnormal. CSF glucose less than 40 mg/dl or two-third of blood sugar was considered abnormal. If LP was not traumatic, the presence of RBC in CSF was considered abnormal.

As standard care of the sick newborn admitted in the hospital, all critically ill neonates in the pediatric ward were treated by ampicillin and gentamycin as the first and empiric antibiotic regimen. In cases with positive cultures of CSF or blood, antibiotics were changed based on the antibiogram. In cases with a high index of suspicion for meningitis, the third generation of cephalosporin (cefotaxime) is added to the antibiotic regimen.

The variables such as gestational age, birth weight, and gender, mode of delivery, head ultrasound findings, and CSF values were evaluated and compare

between two groups.

This study was performed according to the Helsinki Statement and the Ethics Committee of the Shahid Beheshti University of Medical Sciences. The information was collected in an anonymous way using the coding system.

For statistical analyses, the continuous variables were characterized by mean, standard deviation, inter-quartile range, and ninety-fifth percentile. Qualitative variables were described by the frequency. CSF findings were compared in term and preterm infants, based on gender, birth weight, and age during the LP procedure, and type of delivery. All statistical analysis was performed by the SPSS v.22.0 (IBM Corp., Armonk, NY, USA).

Results

In this study, 248 neonates have enrolled which 110 neonates were females (44.4%) and 138 were males (55.6%). Fifty-five percent of them were term and 45% were preterm. The mean birth weight in term babies was 3409 ± 65 gram and in preterm neonates was 1373 ±376grams.

Table 1 showed the demographic data of the patients in two groups. As represented, the mean gestational age of the patients in the preterm and term group was: 31.66 and 38.46 weeks. (P value<0.001). The mode of delivery was normal vaginal delivery (NVD) and cesarean section(C/S) in 28.5% and 71.5%in term neonates and 1.8% and 98.2% in premature infants (P value<0.001) with a significant increase the rate of C/S in preterm babies. The mean age of patients at the time of lumbar puncture was: 25.96 and 9.66 days in term and preterm neonates with statistically significant difference between two groups (P value<0.001).

Table 2 compare the levels of CSF markers in term and preterm neonates. As represented, the mean level of protein(mg/dl), sugar(mg/dl), WBC (cells/µl), PMN, LYM and RBC(cells/µl) in term and preterm infants was: 10.23 Vs. 71.47, 54.47 Vs.57.27, 19.60 Vs.16.43, 54.36 Vs. 45.48, 36.55 Vs.36.90, 17096 Vs.6059 respectively. There was no statistically significant difference between all mentioned items except for the protein level, in which preterm babies had higher levels of protein in their CSF in comparison with term neonates (P value<0.001).

Table1. Demographics data of the study population.

Variable	Term or preterm	Numbers	Mean	Standard deviation	p-value
Age (days) at the time of admission	Term	137	8.63	6.82	0.018
	Preterm	111	13.52	20.56	
Gestational age (weeks)	Term	137	38.46	1.20	<0.001
	Preterm	111	31.66	2.65	
Age (days) at the time of lumbar puncture procedure	Term	137	9.66	7.45	<0.001
	Preterm	111	25.96	16.41	
Birth weight (gr)	Term	137	3409.08	650.55	<0.001
	Preterm	111	1373.33	376.94	
Gender	Female	53 (38.7)	57 (51.4)		0.062
	Male	84 (61.3)	54 (48.6)		
Delivery type	Normal vaginal delivery	39 (28.5)	2 (1.8)		<0.001
	Caesarian section	98 (71.5)	109 (98.2)		

Table2. Comparison of CSF values in term and preterm infants.

Variable	Term or preterm	Numbers	Mean	Standard deviation	p-value
CSF glucose (mg/dl)	Term	137	54.48	23.54	0.037
	Preterm	111	57.27	25.41	
CSF protein (mg/dl)	Term	137	10.23	45.27	<0.001
	Preterm	111	71.47	127.47	
Number of CSF WBCs (cells/mm^3)	Term	137	19.60	71.93	0.723
	Preterm	111	16.43	67.79	
Percentage of CSF PMNs	Term	22	54.36	32.10	0.374
	Preterm	21	45.48	32.78	
Percentage of CSF lymphocytes	Female	22	36.55	29.33	0.969
	Male	21	36.90	30.84	
Percentage of CSF RBCs	Normal vaginal delivery	137	17096.84	87473.02 26136.41	0.163
	Caesarian section	111	6059.96		

CSF= Cerebrospinal Fluid, WBC= White Blood Cell, PMN= polymorphonuclear leukocytes, RBC= Red Blood Cells

Table 3 compare the percentages of abnormal CSF markers between two groups. Overall, in the analysis of the CSF, the protein, glucose, WBC, PMN, and RBC were normal in 92.7%, 79.4%, 89.9%, 91.5%, and 35.5% of cases, respectively.

The percentages of abnormal CSF parameters in the two groups did not differ significantly.

Regard to the comparison of the CSF analysis of preterm neonates with term babies, we evaluated the CSF parameter of 112 term neonates. There was a statistically significant difference between the two groups in the age of admission (P-value: 0.018), gestational age (P-value :< 0.001), birth weight P-value :< 0.001), the age of neonate at the time of LP (P-value :< 0.001).

In addition to other variables, the number of normal values of CSF protein, glucose, WBCs, PMNs, and RBCs was compared between term and preterm infants. The percentages of abnormal CSF parameters (protein, sugar, WBC, PMN, LYM, and RBC) were similar between the two groups with no statistical difference. (P-values were 1, 1, 0.770, 1 and 0.192, respectively (Table 3).

Overall, meningitis was diagnosed in 10.1% of all eligible cases. (9% of preterm infants and 10.9% of term neonates). Given that all preterm neonates admitted to the NICU evaluated for IVH by ultrasonography at bedside on the other hands IVH can changes the CSF parameters such as RBC, sugar and protein, in our research we analyzed the results of brain sonography of the patients. we found that ultrasound findings were normal in 72% of cases, intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) was found in 23.7% and 3.4% respectively, however high grade of IVH which confound the results of research, excluded the study.

Table3. Comparison of abnormal CSF markers between term and preterm infants.

Variable		Term N (%)	Preterm N (%)	p-value
CSF protein	Normal	127 (92.7)	103 (92.8)	1
	Abnormal	10 (7.3)	8 (7.2)	
CSF glucose	Normal	109 (79.6)	88 (79.3)	1
	Abnormal	28 (20.4)	23 (20.7)	
CSF WBC	Normal	122 (89.1)	101 (91)	0.770
	Abnormal	15 (10.9)	10 (9)	
CSF PMN	Normal	122 (89.1)	101 (91)	1
	Abnormal	15 (10.9)	10 (9)	
CSF RBC	Normal	54 (39.4)	34 (30.6)	0.192
	Abnormal	83 (60.6)	77(69.4)	

CSF= Cerebrospinal Fluid, WBC= White Blood Cell, PMN= polymorphonuclear leukocytes, RBC= Red Blood Cells

Discussion

Regarding the increase of the survival rate of very preterm infants in these days, the complications of prematurity is increasing. Neonatal sepsis and meningitis are the two most common consequences of preterm delivery and prolonged NICU admission of these babies. Given that the normal values of CSF markers (sugar, protein and WBC, RBC) of neonates are different from the older ages, it is likely that the normal values of CSF markers of preterm babies are different with term neonates. In this research, we compare the CSF markers of term and preterm infants. As we have shown, the sugar and number of CSF WBCs are not different in terms and preterm infants (the difference is not statistically significant). But it was shown that CSF protein levels in preterm infants are significantly higher than those in terms (10.23mg/dl vs. 71.47 mg/dl, P-value <0.001). The age of neonates at the time of LP was lower in term neonates, which is because the term infants who are diagnosed with sepsis and meningitis undergo LP at an earlier age. Considering that in most cases of preterm infants in the early days of life respiratory distress syndrome, mechanical ventilation, and instability of the hemodynamic condition of patient, does not let the procedures such as LP in neonates, this different between the age of neonates at the time of LP in term and preterm infants are justified. Our data revealed that 92.7% of neonates had normal protein level while just 35.5% had normal RBC count in the CSF. This analysis showed the high number of traumatic LP in neonatal population. Since the Mofid Children's' hospital is a training center and the procedure of LP was done by pediatrics residents of neonatal-perinatal fellowship, high percentages of LP is normally expected. The percentages of normal glucose, WBC and PMN of CSF were in 79.4%, 89.9%, 91.5%, and 35.5% of cases respectively.

Although some previous studies have defined normal values of CSF in neonates, there is still a lot of controversy in this area. Normal CSF values are important for the diagnosis of central nervous system infection in cases where CSF culture may have false-negative results. Most studies that have been done to determine reference values are retrospective with a small population of infants [7, 13, and 14]. Most studies did not have appropriate exclusion criteria. Some infants with traumatic LP, bacteremia, and seizure have not been excluded from some studies [6, 13, and 14]. Also, only a small percentage of infants

evaluated in these studies were preterm [15, 16].

As mentioned earlier, our study showed that there is a significant difference between CSF protein levels in term and preterm infants (P values <0.001). This finding of our study is similar to study by Srinivasan et al in 318 infants consisted of 148 (47%) preterm neonates and 170 (53%) term babies. This research revealed that the level of protein of CSF in premature neonates was more than term babies (209 mg/dL in preterm neonates vs. 159 mg/dL in term infants; P < .001), but WBC (12 cells/μL vs.14 cells/μL) and sugar were similar between two groups [17].

This difference between the protein level of CSF between term and preterm babies, would probably be due to greater permeability in the blood-brain barrier in preterm infants [18]. Also, the amount of growth factor and other proteins may be present in the infant's CSF [19].

In addition, glucose, WBC count, RBC count, PMN percentage, and lymphocyte percentage were not significantly different between the two groups. This finding confirms other previous studies such as study done by Arijit Majumdar et al in which they revealed significant different level of protein level of the CSF in premature infants compare with term neonates (68.2 vs..72.6 mg/dl) (P<0.05)and similar levels of sugar (P = 0.5) and WBC(P = 0.6) [18,20]. But some other recent retrospective analyzes reported differences in CSF values in term and preterm infants [21]. Contrary to their results, we did not find the difference in the number of WBCs and the concentration of glucose between term and preterm infants.

However, the incidence of meningitis in preterm babies is more than term neonates, but the rate of meningitis in term infants was more than premature ones. Considering the small sample size of our study, this puzzling result, does not rule out the importance of prematurity as a risk factor for sepsis and meningitis. The strength of our study is the relatively large number of infants. Also, the number of missing values in our study was negligible. Given that our hospitals are the referral center for infants from all over the country, the publicity of our findings is also acceptable

Our study has also some limitations. Data were retrospectively derived from the records of hospitalized infants, while it was more reliable if it was done prospectively. The incompleteness of the records caused limitations in the study, which led to the withdrawal of patients and a decrease in the number of participants. We tried to exclude patients with incomplete records to decrease the missing data. Also, very few of our infants were older than 28 days, which makes it difficult to use our results for older infants. We obtained reference values from infants who had been undertaken for suspected sepsis and meningitis. Consequently, the reported values may not reflect a truly healthy population of infants.

Conclusion

In conclusion, CSF protein in preterm infants was significantly higher compared to term ones, but RBC, WBC, and CSF glucose were not significantly different between term and preterm infants. Considering that our study was retrospective and with the limitations mentioned, some prospective studies with follow up, mortality, and neurodegenerative evaluations can better represent the application of CSF values in preterm infants.

Conflicts of Interest

The authors declare that they have no competing interests.

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