Cross-sectional Study

A cross-sectional study on the correlation between blood phosphorus level with sepsis and associated prognostic factors in neonates

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ABSTRACT

Objective: Neonatal sepsis is one of the most critical causes of infant mortality. Alteration in phosphorous levels is known to be associated with sepsis. The aim of this study is to evaluate the level of blood phosphorus in neonates admitted to ICU and its relation with different factors and prognosis of patients.
Methods: In this cross-sectional study, all neonates admitted to the intensive care unit diagnosed with neonatal sepsis were included. Serum phosphorus levels were evaluated along with c-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and urine and blood culture. Demographic data along with clinical findings were collected in a research questionnaire for all the patients.
Results: Of 211 neonates, 98 (46.4%) were female and 113 (53.6%) were male, and the mean age of the patients was 10.51 days. The mean phosphorus level was 4.39 ± 0.67 mg/dL. The mean phosphorus levels among girls and boys was significantly different, $p = 0.001$ (4.23 ± 0.62 vs 4.53 ± 0.69 mg/dL). The mean phosphorus among positive and negative blood culture patients was also significant, $p < 0.001$ (4.74 ± 0.67 vs 4.29 ± 0.64 mg/dL). However, type of feeding, ESR, urine culture and CRP status was not associated with phosphorus levels, $p > 0.05$.
Conclusion: The alterations in phosphorous levels among neonatal sepsis patient is likely to be correlated with gender and blood culture status. Other prognostic markers might not have an effect on phosphorous levels in these patients.

1. Introduction

Neonatal sepsis is a generalized bacterial infection that occurs in the first month of life and is confirmed by positive blood culture in the presence of laboratory clinical findings and is a major cause of neonatal mortality. The prevalence of sepsis has been reported differently in different countries. In developed countries, the prevalence of sepsis is equivalent to 1–4/4000 live births, while in poor and developing countries it is almost ten times higher [1]. Neonatal sepsis is a clinical syndrome that presents as bacteremia in the first month of life with systemic symptoms [2].

The clinical and laboratory signs of neonatal sepsis are often nonspecific [3], but treatment should not be delayed, as failure or delay in treatment can lead to many fatal complications [4,5]. On the other hand, with early diagnosis of sepsis, limited and unnecessary long-term treatments, length of hospital stay and treatment costs are significantly

reduced [6]. Tachycardia, respiratory distress, jaundice, non-breastfeeding status, lethargy and cyanosis have been the most common clinical findings in studies in Iran [7–9]. Numerous studies have been performed for early detection of sepsis and several hematological and laboratory indicators have been suggested for screening for sepsis [10,11].

Phosphate controls a number of processes involved in homeostasis and is regulates by kidney, bones and digestive system [12]. Hypophosphatemia has long been reported with sepsis and is associated with the severity of sepsis [13]. It is also associated with increased admission to intensive care unit and cardiac arrythmias in sepsis patients [14].

Given that sepsis is one of the most important causes of mortality and disability in infancy, with timely diagnosis and treatment of the disease and by improving the quality of care during childbirth and infancy, the survival rate of infants can be increased [26]. It can be expected that serum phosphate levels have an effect on the prognosis of neonatal

sepsis and associated factors [15]. Therefore, the present study investigates the level of blood phosphorus in infants admitted to the intensive care unit and its relationship with various factors as well as the prognosis of patients.

2. Methods

2.1. Study population

This cross-sectional study was performed on neonates who were diagnosed with sepsis and had initiated treatment. All neonate diagnosed with late sepsis were included in the study. Those with birth weight less than 2500 g, intrauterine growth disorders, minor and major anomalies and asphyxia were excluded from the study.

2.2. Diagnosis of neonatal sepsis

Sepsis was confirmed in the patients based on positive blood culture proliferation according to the recommendations of the European Medicines Agency (EMA) criteria indicating the presence of minimum two clinical symptoms or laboratory indications (positive culture, microscopy and/or real time polymerase chain reaction) [16].

2.3. Sampling and data collection

Based on this, blood samples were taken, centrifuged within 6 h of sample collection, and the serum was separated, and then frozen at minus 20° Celsius. In the clinical laboratory of the hospital, blood phosphorus level was measured using calorimetric method (Pars Azmoon commercial kits, Tehran, Iran). Serum CRP (C-reactive protein) levels were measured using ELISA (enzyme linked immunosorbent assay) kits (Diagnostics Biochem Canada Inc, Canada).

To determine the sample size, taking into account $\alpha = 5\%$, S = 2.5 and d = 0.4 and using the following formula, approximately, 200 people were selected in this study.

$$n = \left(\frac{\mathbf{Z}_{1-\sqrt{x}}.\mathbf{S}}{d}\right)^2$$

2.4. Data analysis

After collection, the data was entered in SPSS version 21. Central ratios and indices and the dispersion were calculated and independent *t*-test or Mann-Whitney test were used for comparison. Multivariate linear regression was also used to investigate the simultaneous effect of the studied variables on neonatal blood phosphorus levels. The results were reported at a significance level of 5%.

This study was approved by the Research Ethics Board of (XXX).

2.5. Unique identifying number is: researchregistry7684

The methods were stated in accordance with STROCSS 2021 guidelines [17].

3. Results

3.1. Patients' demographics and characteristics

211 neonates, 98 (46.4%) girls and 113 (53.6%) boys were studied. The mean age was 10.51 $\pm$ 2.17 days. Of these, 129 (61.1%) were breastfeeding, 60 (28.4%) were formula-fed and 22 (10.4%) were breastfeeding and formula-fed. 46 (21.8%) patients had positive blood culture and 165 (78.2%) patients had negative blood culture. Urine culture was positive in 46 (22.1%) patients and negative in 162 (77.9%) patients. 196 (92.2%) patients were CRP positive and 15 (7.1%) patients were CRP negative. ESR (erythrocyte sedimentation rate) in 28 (13.3%)

patients were below 20 mm/h and in 183 (86.7%) patients were more than 20 mm/h (Table 1).

3.2. Correlation of phosphorous levels with gender, blood and urine culture and CRP

Comparison of mean phosphorus levels based on sex, blood culture, urine culture and CRP of neonates using univariate analysis showed that between the mean phosphorus levels among males and females was significantly different, p = 0.001 (Table 2). The mean phosphorus among negative and positive blood culture patients was also significant, p < 0.001. However, urine, ESR, CRP and type of feeding, was not associated with phosphorus levels, p = 0.53, p = 0.33, p = 0.055 and p = 0.483, respectively (see Table 3).

The results of multivariate regression showed that assuming other variables were constant (adjusting for other variables), the phosphorus level of female infants was on average 0.2 units lower than those in boys, which according to p = 0.031, this decrease was statistically significant. Similarly, after adjusting other variables, the level of phosphorus in the blood of neonates with negative blood culture was 0.42 units lower than the neonates with positive blood culture on average, which was statistically significant, p < 0.001 (Table 4). Other studied variables had no effect on neonatal blood phosphorus levels (p > 0.05).

4. Discussion

In the present study, 211 neonates including 98 (46.4%) girls and 113 (53.6%) boys with sepsis admitted to the intensive care unit were studied. The mean age of 211 infants in terms of days was 10.51 and their age range was 7–14 days. 46 patients (21.8%) had positive blood culture and 165 patients (78.2%) had negative blood culture in our study. Furthermore, our study also showed greater prevalence of negative urine culture (77.9%).

In a study conducted by Mehrkhah et al., on 5370 children with clinical symptoms and suspected sepsis, 55% patients were boys and 45% were girls [18] and in the study by Talei et al., out of 102 patients, 60 (58.8%) were boys and 42 (4.2%) were girls [19], which is similar to our findings. In the study by Heydarian et al., the mean age of infants at the time of admission was 8.1 days [20], which is lower than the average age of our study. Keshtkari et al., reported only one case of primary positive blood culture with *Staphylococcus aureus* out of 100 patients [21], and the study by Rahbarimanesh et al. indicated the prevalence of negative blood culture in patients, however, it was suggested that blood culture testing should be performed in suspected cases [22].

Increased CRP due to its short half-life (4–6 h) is helpful in diagnosing the disease and in tracking the effect of antibiotics [18–21]. In the study of Keshtkari et al. 26% cases were CRP positive and in the present study 196 (92.2%) were CRP [21]. An increase in ESR is also reported with sepsis in 30–70% patients through various studies, and

Table 1
Frequency distribution of demographic and clinical characteristics of neonates with sepsis.

		Number	%
Girl	Gender	98	46.4
boy		113	53.6
Breast milk	Type of nutrition	129	61.1
Powdered milk		60	28.4
Mix		22	10.4
+	Blood culture	46	21.8
-		165	78.2
+	urine culture	46	22.1
-		162	77.9
+	CRP	196	92.2
-		15	7.1
<20	ESR	28	13.3
>20		183	86.7

Table 2
Mean demographic and clinical characteristics of neonates with sepsis.

	Domain	Mean ± SD	Number
Age (day)	14–7	2.17 ± 10.51	211
Phosphorus level (mg/dL)	6.2–3.1	0.67 ± 4.39	211

Table 3
Comparison of average phosphorus levels with independent variables (P < 0.05 was considered statistically significant).

sub scale	SD ± Mean	type of employment	P-Value
Gender	0.62 ± 4.23	Girl	0.001
	0.69 ± 4.53	Boy	
Blood culture	0.69 ± 4.74	+	0.001>
	0.64 ± 4.29	–	
urine culture	0.83 ± 4.44	+	0.53
	0.63 ± 4.37	–	
CRP	0.69 ± 4.38	+	0.33
	0.45 ± 4.56	–	
Type of nutrition	0.55 ± 4.30	Breast milk	0.055
	0.82 ± 4.51	Powdered milk	
	0.81 ± 4.57	Mix	
ESR	0.51 ± 4.31	<20	0.483
	0.70 ± 4.4	>20	

Table 4
Analysis of variables of sex, type of nutrition, blood and urine culture, ESR, CRP, and age on neonatal phosphorus levels (P < 0.05 was considered statistically significant).

Variable	Group	Patient error	Confidence Interval	Coefficient B	P-value
Gender	Boy	–	–	Ref	0.031
	Girl	0.09	0.02-,0.39-	0.2-	
Blood culture	+			Ref	<0.001
	–	0.12	0.19-، 0.65-	0.42-	
urine culture	+			Ref	0.44
	–	0.11	0.14، 0.3-	0.09-	
ESR	<20		–	Ref	0.36
	>20	0.14	0.39، 0.14-	0.12	
CRP	+	–	–	Ref	–
	–	0.18	0.62، 0.1-	0.26	0.16
Type of nutrition		0.07	0.25، 0.02-	0.11	0.09
Age		0.02	0.06, 0.02-	0.02	0.32

with the improvement of the disease, it gradually goes back within the normal range [23,24]. In the present study, the ESR of 28 patients (13.3%) was less than 20 mm/h and 183 patients (86.7%) were more than 20 mm/h.

Definitive diagnosis of sepsis is made by isolating the microorganism from a specific source (such as blood, cerebrospinal fluid, urine by sampling through suprapubic and other body fluids). Phosphorus is one of the components of the intracellular environment and proteins, enzymes, transcription factors, lipid and carbohydrate mediators, ATP and nucleic acids which makes it necessary for the body to function [25]. Electrolyte disturbances occur frequently in patients with critical conditions. Hypophosphatemia is one of the electrolyte disorders that can occur in critically ill patients hospitalized for various reasons [26]. Therefore, it can be expected that serum phosphate levels have an effect on the prognosis of neonatal diseases admitted to the neonatal intensive care unit. Normal levels of phosphorus in adults are 2.5–5 mg/dL and in children 3–6 mg/dL. In the 2018 study by Miller et al., out of 197 patients with sepsis, 33 were classified as hypophosphatemia and 41 as hyperphosphatemia [27]. In the present study, the mean phosphorus level of 211 patients was 4.39 ± 0.67 mg/dL. In study by Brener Dik et al., examining the prevalence of hypophosphatemia during the first

week of life in preterm infants on an aggressive injectable diet, the results showed a 91% prevalence of hypophosphatemia. The mean neonatal blood phosphorus was 2.52 mg/dL. The results of this study showed that infants with severe hypophosphatemia (phosphate less than 2 mg/dL) were younger. The prevalence of sepsis was higher in these infants, and the need for vasoactive drugs and mechanical ventilation was higher in them [28], which is inconsistent with the findings of present study. In our study, a comparison of mean phosphorus levels based on sex, blood culture, urine culture and CRP of neonates with sepsis admitted to the intensive care unit showed that there was a significant difference between the mean phosphorus levels of male and female neonates (P = 0.001). There was no significant difference between the mean neonates with positive and negative blood culture (P < 0.001) and in other cases (urine culture, type of feeding, CRP and ESR), P > 0.05. Also, the correlation between age and phosphorus level of neonates was not statistically significant according to P = 0.2.

In a study by Shor et al., severe hypophosphatemia in sepsis was performed as a mortality predictor. Fifty-five patients were selected and divided into two groups: Group 1 included 26 patients with severe hypophosphatemia (serum mineral phosphate (Pi) < 1 mg/dL) and group 2 included 29 patients without severe hypophosphatemia (Pi > 1 mg/dL). Phosphorus levels did not have a significant relationship with age and sex [29]. The results also showed that 80.8% of patients with severe hypophosphatemia died, indicating significance in the relationship [29]. Antachopoulos et al. conducted a study on transient hypophosphatemia associated with acute infectious diseases in children. A significant negative correlation was observed between serum phosphate and CRP level (r = −0.41, p < 0.0001). Patients with CRP≥ 151 mg/L had lower mean serum phosphate at the time of admission than patients with CRP≤ 50 mg/L (1.17 vs. 1.50 mmol/L, P < 0.0001). The overall prevalence of hypophosphatemia in patients with pneumonia, upper respiratory tract infection, bacterial urinary tract infections and viral infections were 45%, 35.7%, 18% and 4.4%, respectively. The findings of this study indicated that hypophosphatemia is associated with increased CRP levels and resolves before CRP levels return to normal. As a result, hypophosphatemia is a relatively recurrent but is transient phenomenon in children with acute infectious diseases [30]. The level of CRP with phosphorus is significantly different from the present study.

Our study does not include controls and complications and comorbid conditions were not evaluated in the study. Further studies are therefore required in this area to confirm these findings. Diagnostic efficiency of different measurement kits can vary which can lead to discrepancies in results.

Our study showed that hypophosphatemia is significantly more in girls and patients with negative blood culture.

5. Conclusion

Our study showed a significant correlation between the mean phosphorus levels of neonates in terms of sex, blood culture, urine culture and CRP. The phosphorous levels of female infants in likely to be lower than males and those with negative blood culture. Other variables, any not have effect on phosphorous levels of these infants.

Ethical approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Contributors' statement page

Dr. Hamidreza Sherkatolabbasieh: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. Majid Firouzi: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr. Mahshid Garmsiri: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Conflict of interest

The authors deny any conflict of interest in any terms or by any means during the study.

Registration of research studies

1. Name of the registry: N/a
2. Unique Identifying number or registration ID: **IR. LUMS. REC.1398.268.** Hyperlink to the registration (must be publicly accessible):https://ethics.research.ac.ir/ProposalCertificateEn.php?id=123047&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true.

Guarantor

Dr. Majid Firouzi.

Consent to participate

From the under 16 years old was given by a parent or legal guardian.

Consent for publication

Not applicable.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103582.

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