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Original Article

Serum zinc level and prognosis of neonatal sepsis

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Abstract

Background The prognosis of neonatal sepsis can be influenced by various risk factors, one of which is a deficiency of zinc micronutrient substances.

Objective To assess for a potential association between serum zinc level and prognosis of infants with early-onset neonatal sepsis (EONS).

Methods This prospective cohort study was done in neonates with clinical EONS from September 2017 until December 2018. Serum zinc level was measured on the first day of diagnosis and prognosis was assessed on the fourth day. The association between serum zinc levels and prognosis of EONS was analyzed by Chi-square test and logistic regression with adjustment for confounding variables.

Results A total of 70 subjects were divided into two groups based on their serum zinc levels. A cut-off point of 75 ug/dL was used based on area under the curve (sensitivity 91.2% and specificity 93.7%), with accuracy 97.8% (95%CI 0.943 to 1.000; P=0.0001). Subjects with low zinc level had a 16.8 times greater risk compared to subjects with high serum zinc (RR=16.81; 95% CI 4.35 to 65.02; P <0.0001). Multivariate analysis revealed that subjects with low serum zinc levels had 203.7 times greater risk of worsening than subjects who had a higher serum zinc level (RR 203.72; 95% CI 26.79 to 1549.17; P <0.0001). Covariates such as male sex, low gestational age (<37 weeks), low birth weight (<2,500 grams), asphyxia, Caesarean section delivery, and the presence of comorbidities did not have significant associations with outcomes of EONS (P >0.05).

Conclusion Serum zinc level is associated with prognosis of early onset neonatal sepsis, with a cut-off of 75 μg/dL. The high level of serum zinc associates with a better prognosis. **[Paediatr Indones. 2020;60:37-42; doi: http://dx.doi.org/10.14238/pi60.1.2020.37-42**].

Keywords: *serum zinc levels; neonatal sepsis; prognosis; early-onset neonatal sepsis*

Neonatal sepsis is a major problem that requires special attention in the field of neonatal care because of its high prevalence and mortality. The diagnosis and management of neonatal sepsis are often late and various risk factors aggravate the condition. One of these factors is a neonatal deficiency in zinc micronutrient substances which may lead to poor prognoses.

Sanglah Hospital, Denpasar, Bali, the referral hospital on the island of Bali, had 458 suspected cases of sepsis in 2008-2009, and a prevalence of neonatal sepsis of 5.3% in 2004.[1] The incidence of neonatal sepsis in 2010 was 5.0% of patients treated. Based on preliminary data obtained from the Sanglah Hospital annual reports from 2013 and 2014, the prevalences of neonatal sepsis treated in the Neonatology Ward were 170 (15.8%) and 225 (22.0%), respectively. The mortality rate at Sanglah Hospital in 2010 was 30.4%, which means that even though the incidence of treated sepsis was low (5 per 100 patients), a

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high mortality rate of 30 out of 100 patients treated occurred.[2]

The time of sepsis onset can be a risk factor for poor prognosis, as in early-onset neonatal sepsis (EONS), which is sepsis occurring at under 72 hours of age, compared to late-onset neonatal sepsis (LONS), occurring at over 72 hours of age. Early onset neonatal sepsis is the most common cause of morbidity and mortality in premature infants.[3,4] Differences in etiologic agents have a bearing on the prognosis and long-term complications in septic newborns. In addition, deficiencies of micronutrients in the body, such as iron (Fe), copper (Cu), vitamin A, zinc, and other minerals, can aggravate sepsis. Zinc can affect prognosis in neonatal sepsis, as low zinc levels in septic neonates were noted to have a worse prognosis.[5] Various studies on zinc levels in neonates reported the average serum zinc level of healthy neonates was 75.0–98.0 μg/dL,[6] while serum zinc deficiency was considered to be <65.0 μg/dL.[7] A previous study noted decreased serum zinc levels in neonatal sepsis, which worsened in more severe sepsis.[8]

A study found that mean serum zinc levels in children with clinical symptoms of infection [45.0 (SD 13.7) μg/dL] differed from those without clinical symptoms of infection [49.0 (SD 13.7) μg/dL], but the difference was not signficant.[9] In another study, serum zinc levels in children infected with positive laboratory results (increased CRP or leukocytosis) were significantly lower compared with patients with negative laboratory results.[10]

A study showed that serum zinc levels could be used as an indicator of prognostic outcome in neonatal sepsis, in a study of 41 neonatal sepsis cases. Among the lower serum zinc group, 75.0% of septic neonates worsened, while among normal or higher serum zinc groups, only 6.9% of cases had deteriorating sepsis outcomes.[11] This study aimed to assess for a potential association between serum zinc level and prognosis of infants with early-onset neonatal sepsis (EONS).

Methods

This was a prospective cohort design study conducted in the Neonatology Ward at Sanglah Hospital, Denpasar, Bali, from September 2017 until December 2018. The inclusion criteria were neonates with clinically EONS, gestational age >32 weeks, birth weight >1,500 grams, and whose parents provided written informed consent.

The exclusion criteria were neonates with major congenital anomalies, gastrointestinal infection/disorders, multiorgan failure, or malignancy. We used a consecutive sampling method. The required minimum sample size was based on unpaired, categorical, comparative analytics, with alpha 0.05 and power 0.8, and calculated to be 33 subjects per group. Serum zinc levels was measured in venous blood specimens when clinically EONS was diagnosed. The zinc measurement was carried in the laboratory, using the inductively coupled plasma-mass-spectrometry (ICP-MS) method. Zinc levels were divided into high and low, based on the cut-off values obtained from the area under the curve (AUC) analysis. The high serum zinc level if it was above the cut off value and the low serum zinc level if it was below the cut off value. Serum zinc levels are expressed in units of ug/dL.

Clinically EONS was defined as a condition in which signs and symptoms of neonatal sepsis were found based on clinical and laboratory results (possible/probable sepsis). The clinical signs and symptoms consisted of lethargy, convulsions, whimpering cry, high pitch cry, weak suction reflexes, convex crown, hypotonic, cyanotic/pale/yellow skin, cold acral, sclerema, edema, abnormal temperature, abnormal breathing rate, abnormal pulse rate, vomiting, abdominal distension, splenomegaly, decrease of urine volume, bleeding, and shock. The laboratory results including IT ratio value >0.2, total number of PMN cells <1800/mm^3 or >5400/mm^3, leukocyte count <5000 or >30000/mm^3, and platelet count <150000 or >360000/mm^3. If there were only 3 clinical symptoms out of 6 clinical signs and symptoms groups, the subject was classified as possible sepsis. If the subject had 3 clinical signs and symptoms with laboratory abnormalities, he/she was categorized as propable sepsis.

Prognosis of EONS was defined as neonatal sepsis outcomes on the 4th fourth day of treatment. The good outcomes was defined as an improvement of clinical and laboratory findings, while the bad outcome was the worsening of clinical and laboratory findings. Data were analyzed using *SPSS software*. Data are presented in narrative and table format. Serum zinc cut-off point was defined using the receiver

operating characteristics (ROC) curve. Bivariate analysis was done by Chi-square test and multivariate analysis by logistic regression analysis. The level of significance was based on P values <0.05. This study was approved by the Research Ethics Committee of Universitas Udayana Medical School/Sanglah Hospital, Denpasar.

Results

There were 75 neonates with clinically EONS during the study period. Three neonates were excluded because of major congenital anomalies and two for necrotizing enterocolitis. The total subjects comprised of 70 neonates. The subjects underwent venous blood sampling for serum zinc examination on the day of diagnosis. All subjects were followed until the fourth day after diagnosis, at which time they were assessed for EONS prognosis.

Characteristics of subjects are shown in **Table 1**. Serum zinc levels <75 μg/dL were found in 33 subjects (47.1%) and serum zinc levels >75 μg/dL were found in 37 subjects (52.9%). The majority of the subjects (62.9%) were male, with full term gestational age (61.4%) and had normal birth weight (52.9%). The most common comorbidity was neonatal pneumonia (41.4%). The association between serum zinc levels and EONS prognosis was analyzed with Chi-square test. Subjects were categorized into two groups with a serum zinc cut-off point of 75 ug/dL based on AUC, with 91.2% sensitivity, 93.7% specificity, and 97.8% accuracy (95%CI 0.943% to1.000%; P=0.0001).

Bivariate analysis revealed that subjects with serum zinc level <75 μg/dL had a 16.8 times greater

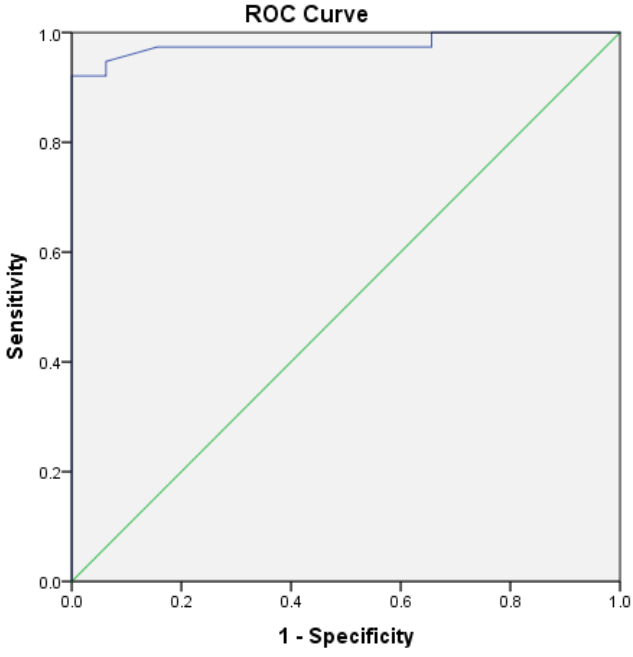

Figure1. Receiver operating characteristic (ROC) curve of serum zinc level

Table 1. Characteristics of subjects

Characteristics	Serum zinc level (N=70)	
	<75 μg/dL (n=33)	>75 μg/dL (n=37)
Gender, n		
Male	20	24
Female	13	13
Gestational age, n		
32- 36 weeks	11	16
>37 weeks	22	21
Birth weight, n		
1,500-2,499 grams	15	18
>2,500 grams	18	19
Asphyxia, n		
Yes	7	14
No	26	23
Birth method, n		
Caesarean section	9	11
Vaginal	24	26
Comorbidities		
Neonatal pneumonia	16	13
Hyaline membrane disease	4	6
Congenital heart disease	6	4
Intracranial bleeding	9	2
Neonatal jaundice	5	6
Hypoxic ischemic encephalopathy	1	4
Chorioamnionitis, n		
Yes	10	9
No	23	28
Blood smear, n		
Normal	21	27
Infection	12	10
White blood cells		
5,000-30,000/ mm^3	11	18
<5,000 or >30,000/ mm^3	22	19
Platelet count		
150,000-360,000/ mm^3	24	25
<150,000 or >360,000/ mm^3	9	12
IT ratio		
<0.2	13	21
>0.2	20	16

risk of deterioration compared to subjects with serum zinc level >75 μg/dL (RR=16.81; 95%CI 4.35 to 65.02; P <0.0001) (**Table 2**). **Table 3** shows the multivariate analysis of various potential contributing factors including serum zinc levels and EONS prognosis. Subjects with low serum zinc levels had a 203.7 times greater risk of worsening prognosis than subjects with high serum zinc level (RR=203.72; 95%CI 26.79 to 1549.18; P <0.0001). Furthermore, covariates such as male sex, low gestational age (<37 weeks), low birth weight (<2,500 grams), asphyxia, Caesarean section, and the presence of comorbidities, did not have significant associations with poor prognostic outcomes from EONS.

Discussion

In our study, zinc levels had a significant relationship with EONS prognosis. The ROC curve revealed that zinc level can be a useful predictor of outcomes in EONS cases, with an AUC value that was close to 1 (97.8%; 95%CI 0.943 to 1.000; P=0.0001). The serum zinc cut-off value was 75 μg/dL, with 91.2% sensitivity and 93.7% specificity. A previous study on 41 neonates with sepsis reported an AUC value of 0.93 (P<0.001) as well as a serum zinc cut-off value of 192.5 μg/dL based on ROC analysis, with 81.8% sensitivity and 90.0% specificity. Neonates with serum zinc level <192.5 μg/dL had a 8.18 times greater risk for worsening sepsis compared to those with higher zinc levels.[11] The different serum zinc levels, and consequently, cut-off points found in our study and that of previous study could be attributed to different inclusion criteria. The previous study used a sample of neonates with gestational age and birth weight greater than ours, and included full term babies with normal birth weight.[11]

The bivariate analysis showed that the serum zinc level in the poor prognosis group was significantly lower compared to the group that experienced improvement. Subjects with serum zinc level <75 μg/dL had a 16.8 times greater risk of deterioration compared to subjects with serum zinc level >75 μg/dL (RR 16.81; 95%CI 4.35 to 65.02; P <0.0001). Similarly, a study found lower zinc levels in conditions of severe sepsis in children aged 1-11 years.[9] Our multivariate analysis showed that subjects with low serum zinc level had a 203.7 times greater risk of worsening than subjects who had higher serum zinc level (RR 203.72; 95%CI 26.79 to 1549.17; P <0.0001).

Another study found that neonates with greater gestational age and weight tended to have higher serum zinc levels.[12] The serum zinc level obtained in each study can be different, this is also due to the influence of nutritional status, population, and techniques in taking and processing blood samples in

Table 2. Bivariate analysis of serum zinc level and EONS prognosis

Variables	Prognosis		RR	95%CI	P value
	Poor	Good			
Serum zinc level					
<75 ug/dL (n=33)	30	3	16.81	4.35 to 65.02	<0.0001
>75 ug/dL (n=37)	2	35			

Table 3. Multivariate analysis of serum zinc levels and EONS prognosis

Variables	Exp (B)	95% CI	P value
Serum zinc <75 ug/dL	203.72	26.79 to 1549.18	<0.0001
Male sex	1.39	0.19 to 9.94	0.744
Gestational age <37 weeks	0.83	0.05 to 14.15	0.899
Low birth weight	1.31	0.09 to 20.02	0.847
Asphyxia	1.34	0.16 to 11.01	0.783
Caesarean section	0.51	0.06 to 4.10	0.526
Comorbidities	2.24	0.23 to 22.20	0.490

each laboratory. However, there were no data showing normal zinc levels for gestational age and infant birth weight.

A study used copper/zinc ratios in EONS patients and showed that the Cu/Zn ratio in infected neonates was relatively higher, indicating that zinc levels acting as denominators in the ratio formula tended to decrease. Statistical tests showed that plasma Cu levels in neonates with sepsis were not significantly different from that of controls (neonates without sepsis) (P=0.177), while zinc levels were significantly lower than that of controls (neonates without sepsis) (P <0.001). They also found that plasma zinc concentrations did not have significant correlations with other variables such as gender, gestational age, or birth weight in neonates with a diagnosis of EONS.[13]

We also found that low serum zinc was significantly associated with the outcome of sepsis. On multivariate analysis, covariates such as male sex, <37 weeks gestational age, birth weight <2,500 grams, asphyxia, Caesarean section, and the presence of comorbidities, did not have significant associations with outcomes from EONS (P >0.05). Since only zinc level had an association with worsening sepsis prognosis in our study, it was likely not influenced by variables shown to be not significant by multivariate analysis, and the similar proportions between these groups.

The results of multivariate regression analysis using the independent variable selection model and its effect on the dependent variable, resulted in an R-squared value of 78%. The remaining 22% would account for other variables that were not analyzed, such as other factors that can affect the prognosis of sepsis. Such factors may include the lack of other micronutrient substances such as iron (Fe), copper (Cu), vitamin A, and other minerals not examined in this study and also the etiologic agents was not analyzed.

A previous study showed that mortality nine months or more after sepsis diagnosis among neonates with zinc levels below 70 μg/dL was 86.4%, whereas in groups with zinc levels >100 μg/dL at diagnosis, the mortality was only 14.8%.[14] Various studies showed evidence that low serum zinc levels can predict a poor outcome in sepsis.[13,14] The reason is related to the clinical manifestation of zinc deficiency, which is characterized by decreased immune cell function and increased incidence of infection. These manifestations were attributed to changes in immune system function, such as decreased function of B and T cells, decreased phagocytosis, and decreased production of cytokines.[13]

Compared to studies using zinc as a biomarker and predictor factor, more studies have been done to assess the role of zinc in sepsis through the effects of zinc supplementation. The role of serum zinc in neonates with EONS is also evidenced by the effectiveness of zinc supplementation. Zinc supplementation can increase the production of cytokines by helper T lymphocytes which promote cellular proliferation and differentiation. Zinc also increases the production of tumor necrosis factor-alpha (TNF-α) by monocytes, which in turn promotes phagocytotic capability.[15] Several studies have shown a significant role of zinc supplementation in reducing the mortality rate of EONS patients. A meta-analysis by Zhijun et al. showed that zinc supplementation of 10 mg/day in neonates reduced mortality (RR 0.48; 95% CI 0.25 to 0.94) by significantly increasing serum zinc levels (mean difference 81.97 μg/dL; 95% CI 34.57 to 129.37; P=0.0007) after administration for two years.[15]

A caveat of this study is that we could not fully control for other factors that could affect deterioration of sepsis, namely, the presence of other types of micronutrients that were not examined such as iron (Fe), copper (Cu), vitamin A, and other micronutrients. Further study with broader risk factor analysis is needed to evaluate the possible independence of association of serum zinc levels with EONS prognosis. In conclusion, serum zinc level is associated with the prognosis of early onset neonatal sepsis, where serum zinc levels <75 μg/dL are signficantly predictive of a worse prognosis than serum zinc levels >75 μg/dL.

Conflict of Interest

None declared.

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