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CORRELATION BETWEEN SERUM ZINC LEVELS AND SEPSIS OUTCOME IN CHILDREN

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ABSTRACT

Various studies suggest that there has been an increase in the number of cases of sepsis in children in the last two decades, which may be associated with increased survival and morbidity, especially in children. Children living in low-income countries with malnutrition become a great population that is vulnerable to sepsis. Zinc is one of the trace elements that can modulate innate and adaptive immune system. It was believed zinc could be a biomarker to predict sepsis outcome as well as zinc supplementation for reducing sepsis severity. This observational analytic study was conduct in RSUP Prof. Dr. R. D. Kandou Manado for four months with total samples of 57 children aged 1 to 18 years old. The result showed that there was a significant relationship between zinc levels and increase morbidity in children (p-value < 0.001). Zinc serum levels at the time of diagnosis of sepsis can be used as an indicator of the outcome of sepsis in children.

Keywords: Sepsis, Zinc, children

INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by a dysregulated immune response to infection.[1] There are many infections that can progress to sepsis, a serious and dangerous clinical condition characterized by organ dysfunction and difficult to diagnose. Sepsis is associated with high morbidity and mortality and was recorded for 23.7 billion health facility expenses in 2013.[2] The Sepsis Prevalence Outcomes and Therapies (SPROUT) study in 2015 collected PICU data from 26 countries, obtained data on the reduction in the global prevalence of severe sepsis (Case Fatality Rate) from 10.3% to 8.9% (95% CI), the mean age of patients with severe sepsis was 3 years, most infections came from the respiratory system (40%) and 67% of cases had multi-organ failure. The incidence of sepsis was higher in neonates and infants < 1 year. Infection that occurs initially will activate the proinflammatory response of the innate immune system. In sepsis, there will be a dysregulation of cytokine responses.

Nutritional factors are important in the management of sepsis. Nutrition is a component in food that is used for the survival and growth of cells.[3] Trace elements are one of the nutrients needed in large quantities for growth. Zinc is one of the key elements that can limit mitochondrial dysfunction that occurs due to the imbalance between reactive oxygen species and antioxidants that occurs in sepsis. The levels of these nutrients decrease in the event of infection because they are used for the formation of antibodies and complement in the acute phase reaction. The more severe the severity of sepsis, the lower the levels of these elements in the body.[4,5]

Zinc deficiency reduces the innate and adaptive immune system. However, the mechanism of the relationship between zinc regulation and the immune system is still unclear. Zinc deficiency causes thymic atrophy, lymphopenia, and reduces adaptive and innate immune responses, interferes with phagocytosis, intracellular elimination activity, interferes with cytokine production by macrophages and body defense by neutrophils and NK cells, interferes with cell proliferation, cytokine production, and B cell antibody secretion and T.[5]

Various studies on reducing zinc levels in seriously ill children have been carried out in which zinc in the erythrocyte membrane can be a sensitive biomarker although this was not seen in several other studies. Nonetheless, zinc as a biomarker has insufficient validity and has not been widely used.[1,2] Several studies have shown the benefits of zinc supplementation in reducing the incidence of infection and length of stay in hospital, but no assessment has been conducted to assess the benefits of zinc supplementation with the incidence of sepsis in children.

Therefore, this study aims to evaluate whether there is a relationship between zinc serum levels and sepsis outcomes.

RESEARCH METHODS

The research method used was observational analytic research with prospective cohort approach in all cases of sepsis in children. This study was conducted in Pediatric Intensive Care unit Prof. Dr. R. D. Kandou Manado Hospital within four months from December 2021 until March 2022 with total sample of 57 children. The inclusion criteria include patient aged 1 month to 18 years old that meets criteria for diagnosis of sepsis. On the other hand, the exclusion criteria include malnutrition and parent refusal for this research. This study was approved by Kandou Hospital Research and Ethics Committee and informed consent was obtained from parents. Sampling was done by consecutive sampling that met inclusion criteria.

Univariate analysis was present with distributive table and parametric data were present in median and standard deviation. Bivariate analysis was using *independent t-test* was used in comparison between two groups for quantitative data and *Mann-Whitney test* was used in

comparison between two groups with non-parametric distribution. The confidence interval was set to 95% and the margin of error accepted was 5%, p-value considered significant if p-value < 0.05 and highly significant if p-value < 0.01. This analysis was continued until logistic regression and Pearson correlation to assess the relationship between serum zinc levels and length of treatment.

RESULTS AND DISCUSSION

Sepsis is a life-threatening organ dysfunction, caused by a dysregulated immune response to infection. [1] Sepsis is associated with high morbidity and mortality and was recorded for 23.7 billion health facility expenses in 2013.[2] The Sepsis Prevalence Outcomes and Therapies (SPROUT) study in 2015 collected PICU data from 26 countries, obtained data on the reduction in the global prevalence of severe sepsis (Case Fatality Rate) from 10.3% to 8.9% (95% CI), the mean age of patients with severe sepsis was 3 years, most infections came from the respiratory system (40%) and 67% of cases had multi-organ failure.

Sepsis describes a complex clinical syndrome that occurs when the host's immune system is activated against infection. Proinflammatory cytokines, such as TNF, IL-1, interferon gamma (IFN-γ) work to help cells destroy infecting microorganisms. Thus, the elimination process is more effective, as well as triggering the release of anti-inflammatory cytokines, such as interleukin-1 receptor antagonists (IL-1ra), IL-4, and IL-10. Anti-inflammatory cytokines play a role in stopping the inflammatory process by modulating, coordinating, or repressing excessive responses (feedback mechanisms). Pro-inflammatory cytokines also play a role in the release of nitric oxide (nitric oxide, NO) is important in elimination of pathogens, but another effect of NO is vascular vasodilatation. In sepsis, excess NO production causes vascular dilatation and causes septic shock.[6,7]

When the immune system is not effective at eliminating antigens, the inflammatory process gets out of control and causes organ system failure. This is in accordance with research conducted by Bone9 which states that multiple organ damage is not caused by infection but is the result of systemic inflammation with cytokines as mediators.

Zinc deficiency reduces the innate and adaptive immune system. However, the mechanism of the relationship between zinc regulation and the immune system is still unclear. Zinc deficiency causes thymic atrophy, lymphopenia, and reduces adaptive and innate immune responses, interferes with phagocytosis, intracellular elimination activity, interferes with cytokine production by macrophages and body defense by neutrophils and NK cells, interferes with cell proliferation, cytokine production, and B cell antibody secretion and Q.[5]

Based on previous studies which showed a relationship between serum zinc levels in children with sepsis, this study aims to determine the relationship between serum zinc levels and length of treatment and mortality in children with sepsis. The method of this study was an analytic observational correlation form with a prospective cohort approach in all cases of sepsis in children. The research was conducted in the inpatient room and PICU at Prof. Hospital. Dr. RD Kandou, Manado, from December to March 2022. Examination of research samples was carried out at Prodia's laboratory and Prof. Hospital's laboratory. Dr. RD Kandou, after giving informed consent to the parents by signing the informed consent, they continued to fill out the questionnaire. Children who met the study criteria underwent routine blood tests, serum zinc levels.

Characteristics of Research Samples

The research sample was children aged 1 year to <18 years who were treated in the PICU and Irina E RSUP Prof. Dr. RD Kandou, Manado with a diagnosis of sepsis. Research starts from December 2021 to March 2022. The results of this study obtained 57 children with a

diagnosis of sepsis, consisting of 38 (66.7%) boys and 19 (33.3%) girls. Table 1 shows the characteristics of the research based on gender and Table 2 shows the characteristics of the research based on age.

*Table**1** Distribution of research samples based on gender*

Gender	Total (n)	Percentage (%)
Man	38	66.7
Woman	19	33.3
Total	57	100

*Table**2** Distribution of research samples based on age.*

Age	**Amount (n)**	**Percentage (%)**
1 month - <2 years	37	64,91
≥2 years - < 6 years	6	10.52
≥6 years - < 13 years	6	10.52
≥13 years - < 18 years	8	14.03
Total	57	100

Research by Nosheen N et al in 2014 found that men with sepsis had a 70% greater mortality rate (p=0.048, RR 1.73) compared to women. This higher mortality was associated with different rates of respiratory tract infection and higher IL-6 levels ($p < 0.01$).[8]
The incidence of sepsis is 15% higher in boys than in girls.[70] This can be caused by an imbalance between T helper 1 (Th1) and T helper 2 (Th2) regulations where the protective immunity mechanism is more dominated by Th1 and Th2 is more related with susceptibility to and exacerbation by infection. The hormone testosterone triggers an immunosuppressive effect by reducing the production of IFN-γ and interleukin 4 by Th 2 cells while estrogen triggers a proinflammatory response from Th1 so that boys are found to be more susceptible to infection.[9]

Characteristics of Mortality

The results of this study found 57 children with a diagnosis of Sepsis, who recovered consisted of 44 (77.2%) children while pediatric patients who died with sepsis were found 13 (22.8%) children. Table 3 shows the characteristics of the study based on the mortality rate.

*Table **3** Distribution of resech samples based on Mortality*

	Amount (n)	Percentage (%)
Healed	44	77.2
Die	13	22.8
Total	57	100.0

This result is when compared with the data obtainedIn the pediatric intensive care unit at Cipto Mangunkusumo Hospital (RSCM) in 2009, the presentation rate for sepsis was 19.3% of 502 pediatric patients treated with a mortality rate of 10%.[10]
In Indonesia, the prevalence of sepsis in children aged 0-18 years has not been widely reported. However, according to data obtained at Sutomo Hospital, cases with severe sepsis were 27.08%, septic shock was 14.58%, while the remaining 58.33% only fell in a state of sepsis. Meanwhile, according to Riskesdas RI 2007, sepsis in children has a mortality rate of 20.5%.

Dyah Kanya Wati et al, the prevalence of children who experience sepsis at the age of 0 years to 18 years is 35.7% with a mortality of 30% and those who experience septic shock are 60.7% with a mortality of 47.1%.

Characteristics of Serum Zinc Levels in Sepsis

The results of this study obtained from 57 children with a diagnosis of Sepsis, it was found that the number of children who experienced zinc deficiency was 13 (22.8%) children, while serum zinc levels were normal, 44 (77.2%) children were found. (Table 4)

*Table **4** Distribution of research samples based on Mortality*

Zinc Serum Levels	Amount (n)	Percentage (%)
< 10.7 µmol/L	13	22.8
10.7 - 16.9 µmol/L	44	77.2
Total	57	100.0

Characteristics of Serum Zinc Levels and Length of Treatment in Sepsis

Of the 57 children diagnosed with Sepsis, the lowest serum zinc level was found in Sepsis patients 8.5 with an average of 12.035 and the highest sepsis serum level in children 15. Meanwhile, the longest length of treatment was 1 day with an average of 11.86 days and the longest length of treatment was 31 days in patients with Sepsis . Table 5 shows the characteristic distribution of serum zinc levels and length of treatment in children with sepsis.

Table 5 Characteristic distribution of zinc serum levels and length of treatment in Sepsis patients

	Average	**SB**	**Median**	**Minimum**	**Maximum**
length of treatment	11.86	7,900	10.00	1	31
Zinc levels	12035	1.8000	12,300	8.5	15.0

The lowest zinc serum level in Sepsis patients was 8.5 with an average of 12.035 and the highest sepsis serum level in children was 15.

Research conducted by Cvijanovich et al, showed low serum zinc concentrations in critically ill children, which is related to the degree of inflammation as measured by the levels of C-reactive protein and interleukin-6 as well as the increasing degree of organ failure.[11]

Another study conducted by Heidemann et al in 2013 showed that serum zinc levels analyzed in the first 72 hours in the PICU had a range from undetectable (<0.1 μg/mL) to 2.87 μg/mL and there were 83.9% of children have zinc levels below normal limits. In this study, it was also seen that lymphopenia was more common in children with low serum zinc levels than normal zinc levels (42.5% vs 25.6%).[12]

Another study conducted by Kartheek et al in 2017 showed that zinc deficiency occurs in 68.8% of children with sepsis.

Correlation Between Zinc Levels and Mortality Outcomes

The relationship between zinc levels and mortality was analyzed by simple logistic regression analysis. Logistic regression analysis discussed the relationship between **zinc levels and the probability of death**. These results are obtained as follows (Table 6).

Table 6 Analysis Result of the Correlation between Zinc Levels and Mortality

Model	Regression Coefficient	Significance	Correlation Value	Conclusion
Constant	11,749			
Zinc levels	-1.149	p < 0.001	rpb = -0.606	Accept H1

Therefore the following form of a logistic regression equation:

$$P = \frac{1}{1+e^{-(11{,}749-1{,}149\,Zink)}} \qquad \text{with } p < 0.001.$$

The results in Table 6 stated that there was a significant relationship between zinc levels and the chance of death (p <0.001) in septic children. The results of Point Biserial correlation analysis to determine the strength of the relationship between the two variables obtained R_{pb} = -0.606. These results suggest that there is a strong relationship between Zinc levels and Morality outcomes in septic children.

Graphically, the relationship between zinc levels and the chance of death can be seen in Figure 1. Figure 1 shows that the lower the zinc level in a septic child, the higher the patient's chance of dying.

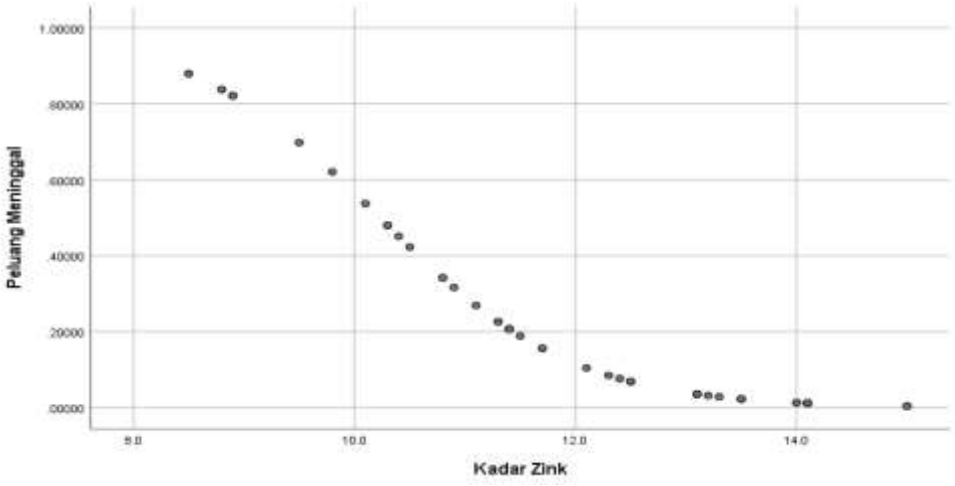

Figure 1 Correlation between zinc levels and the chance of death in septic children

Zinc has an important role in the body's immune function and as an intermediary for various biochemical pathways in the body. Zinc deficiency will interfere with the immune system and cause susceptibility to infection. Zinc deficiency also causes changes in the inflammatory response which are associated with organ damage and higher mortality.[13] Wong et al in 2007 showed that pediatric patients with septic shock who died had low serum zinc levels of 50-55 μg/dL.[14] Besecker et al in 2011 also found high cytokine levels and severity in adult sepsis patients with low serum zinc levels (57.2 ± 18.2 μg/dL).[15] A study conducted by Mertens et al in 2015 showed reduced zinc and selenium levels in septic patients in the intensive care unit with increased oxidative stress and increased inflammatory markers. The zinc and selenium values found in the study were 3.1 (range 1.5–5.4 μM) and 0.42 (range 0.22–0.91 μM).[16]

This study shows that serum zinc levels have a relationship with the stage and ongoing infection process. According to Brown, zinc serum levels decrease at the onset of fever or when the bacterial endotoxin works, known as the acute phase response (2-4 days). The metabolic response to injury and sepsis is early and nonspecific. The acute phase response is mediated by the release of macrophage-activated cytokines, such as interleukin-1 (IL-1) and IL-6, which are released first by monocytes. Macrophages are activated to break down zinc compounds in the liver which are needed for protein synthesis in the acute phase which results in decreased zinc levels in the blood. The decrease in zinc serum levels here only occurs when the infection is severe enough to produce a cytokine response which can eventually be detected in clinical symptoms, and also varies depending on the immune response of each individual neonate. Individual response to sepsis is determined by many factors, including bacterial virulence, blood count, comorbidities, age, and cytokine gene polymorphisms. Decreased zinc levels due to bacterial manifestations are related to the number and duration of presence of bacteria in the blood. The level of suppression of decreased serum zinc levels is also related to the amount of bacterial endotoxin. Several studies on endotoxin levels in infectious processes have shown that large amounts of endotoxins result in exponentially higher levels of circulating cytokines.

Correlation Between Zinc Levels and Length of Treatment

The relationship between zinc levels and length of treatment was analyzed by simple linear regression analysis. This analysis is used because the data of the two variables are in numerical form, as well as Zinc Levels as independent variables (X) and length of treatment as dependent variables (Y).

Based on data analysis, a simple linear regression model was obtained as can be seen in Table 7. The t-test results for the zinc content coefficient obtained t = -0.866 with p = 0.195. These results indicated that there was no significant linear relationship between zinc levels and length of treatment (p = 0.195) in septic children.

Table 7 Results of Analysis of the Correlation between Zinc Levels and length of treatment

Model	Regression Coefficient	t test	Correlation Value	Significance	Conclusion
Constant	17,983				
Rate zinc	-0.509	-0.866	r = -0.116	p = 0.195	Accept H0

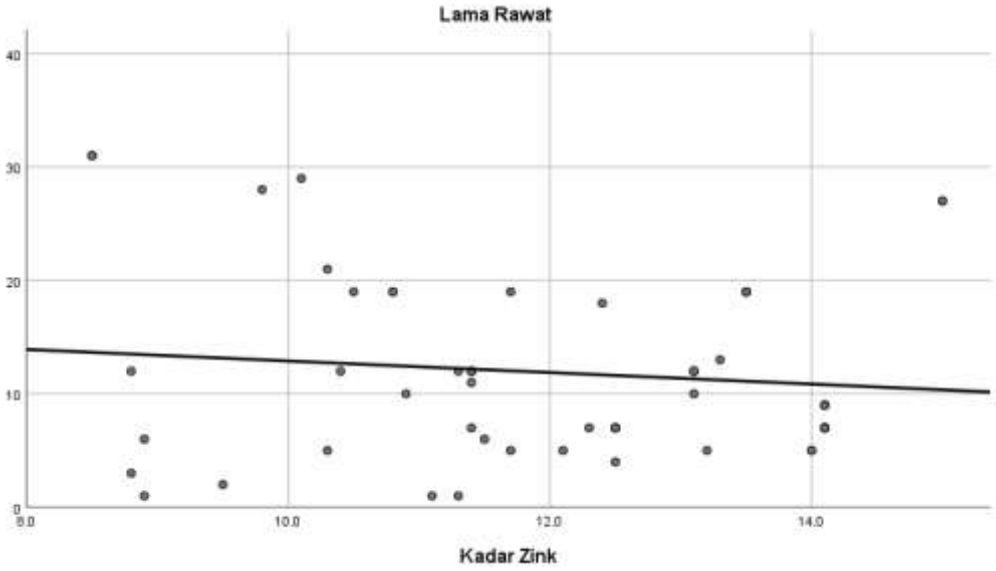

Figure 2 Scatter Diagram of the Relationship between Zinc Levels and Length of Treatment Outcome

The strength of the relationship between the two variables was measured by Pearson correlation analysis. This correlation belongs to the weak category. So for the outcome of the length of treatment in children with sepsis, the higher the zinc level the faster the recovery, but not significant. (Figure 2)

The International Consensus Conference on Pediatric Sepsis, The length of treatment for pediatric sepsis is shortened depending on the severity of sepsis accompanied by the presence of one of: Cardiovascular dysfunction, Acute respiratory distress syndrome and Dysfunction of ≥2 organ systems.

Decreased zinc levels due to bacterial manifestations are related to the number and duration of presence of bacteria in the blood. The level of suppression of decreased serum zinc levels is also related to the amount of bacterial endotoxin. Several studies on endotoxin levels in infectious processes have shown that large amounts of endotoxins result in exponentially higher levels of circulating cytokines.[17]

Ariane's research. A in 2019 a positive predictive value of 75% was obtained, which means that if at the time of examination, the zinc serum level was <29.46 µmol/L, it could be predicted that there would be a 75% worsening. A negative predictive value was obtained at 93.1%, this means that if the serum zinc level is > 29.46 µmol/L then it can be predicted that there will be an improvement in sepsis outcome of 93.1%. [18]

Research Limitations

Researchers are aware of shortcomings in this study, such as small sample size and single entered research conducted in RSUP Prof. dr. R. D. Kandou, Manado. To improve the results of this study, it is hoped that further research can be carried out by including other predictor variables so that the prediction of adverse sepsis outcomes can be more accurate. Furthermore, further research can be carried out to determine zinc supplementation on the outcome of sepsis in children.

CONCLUSION

This study concluded that there is a strong relationship between zinc serum levels and sepsis mortality in children. Lower zinc serum level in septic children could increase the mortality and morbidity of the children. This study also stated that zinc serum levels at the time of diagnosis of sepsis may be used as an indicator of the outcome of sepsis in children.

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