

A new marker for the evaluation of nutrition in pediatric critical care patients: Zonulin

Alper Köker¹, Yasemin Çoban¹, Sultan Aydın², Gökçen Öz Tunçer³, Yılmaz Akbaş³, Tuğçe Tural Kara³, Ahmet Ufuk Kömüroğlu⁴

¹Hatay State Hospital, Department of Pediatric Intensive Care, Hatay, Türkiye

²Hatay State Hospital, Department of Pediatric Hematology and Oncology, Hatay, Türkiye

³Hatay State Hospital, Department of Pediatrics, Hatay, Türkiye

⁴Yüzüncü Yıl University, Department of Biochemistry, Van, Türkiye

Cite this article as: Köker A, Çoban Y, Aydın S, et al. A new marker for the evaluation of nutrition in pediatric critical care patients: Zonulin. Trends in Pediatrics 2023;4(1):37-41.

ABSTRACT

Objective: Zonulin is a biomarker of increased intestinal permeability. Malnutrition is strongly associated with prolonged length of stay, increased infection, and mortality. This study aimed to determine how the serum zonulin level is affected in patients in PICU, and evaluate the relationship between pre-albumin and Vitamin D.

Method: 35 critically ill pediatric patients were included in the study. The control group was formed of 25 healthy children. The albumin, pre-albumin, Vitamin D, and zonulin levels were examined in patients with findings of infection that regressed during ICU follow-up.

The differences between prealbumin levels, Vitamin D, and zonulin were analyzed with the Mann-Whitney-U Test.

Results: The most common reason for admission to the PICU was respiratory failure in 12 patients. The Vitamin D level was determined to have a mean of 28.8 ± 12.3 ng/mL and <29 ng/mL in 9 patients. The albumin level was determined to be mean 3.3 ± 0.6 mg/dL, and the pre-albumin level was mean 17.8 ± 7.4 mg/dL. The serum zonulin levels in critically ill patients were statistically significantly higher than in the control group. The difference between the zonulin levels of patients with pre-albumin values was statistically significant. The zonulin levels of patients with a Vitamin D value <20 were found to be statistically significantly higher than the zonulin levels of patients with a Vitamin D value >20 .

Conclusion: The higher zonulin level in critically ill pediatric patients may be associated with prolonged catabolic processes, exposure to oxidative and hypoxic stress, and bacterial translocation development associated with all of these. The results of the current study showed a statistically significant negative correlation between Vitamin D and zonulin levels. Therefore, the relationship between low Vitamin D values and a high zonulin level may be useful in evaluating chronic malnutrition.

The serum zonulin level selected as a biomarker for the surveillance and management of nutrition in critically ill pediatric patients is not an appropriate marker.

Keywords: Zonulin, malnutrition, pediatric critical care, prealbumin, vitamin D



Correspondence: Alper Köker **E-mail:** kokeralper@gmail.com

Received: 03.02.2023 **Accepted:** 08.03.2023

© 2023 The authors. Published by Aydın Pediatric Society. This is an open access article distributed under the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Malnutrition develops more easily and rapidly in children as they have low nutrient reserves, a fast metabolism, and depletion of energy reserves in a shorter time due to metabolic stress. Acute or chronic malnutrition is seen in 24-55% of patients followed up in pediatric intensive care units (PICU).¹ While malnutrition is determined in some of these patients at the time of admission, others may develop malnutrition secondary to deficient nutrient intake associated with the disease during the treatment process in ICU.² Malnutrition in children with severe diseases is associated with the need for mechanical ventilation, a prolonged period of mechanical ventilation, prolonged length of stay in ICU and hospital, increased infection, and increased mortality.^{3,4}

The patient's body weight must be measured on the first day of admission and followed up with regular measurements throughout hospitalization to evaluate the nutritional status of patients and identify acute changes. The energy requirements of critically ill pediatric patients must be evaluated on an individual basis, and the appropriate amount of energy support must be provided, but it is not easy to determine and measure the energy consumption and manage the appropriate nutritional content and amount in these patients. Various monitoring of nutrition methods such as the follow-up at regular intervals of anthropometric measurements, albumin, pre-albumin, and retinol-binding protein are recommended to obtain the ideal nutritional targets for critical pediatric patients.⁴

Low albumin is known to be a marker of malnutrition. Low pre-albumin is also one of the markers of poor nutrition, but as both albumin and pre-albumin levels are affected by inflammation, neither is accepted as a good marker of poor nutrition.⁵ Therefore, none of these methods and markers have been determined as the ideal method in the follow-up of nutrition.

Zonulin is accepted as a biomarker of increased intestinal permeability, which is to a large extent regulated by tight junctions in the intestinal epithelium, and zonulin has an important role in the regulation of these tight junctions. Zonulin is bound to specific receptors in the intestinal epithelium and is found in the structure of the intercellular tight junctions. Separation of this protein from the tight junctions causes an increase in intestinal permeability. When the sensitively regulated zonulin mechanism is genetically damaged, it can cause intestinal and extra-intestinal, autoimmune, inflammatory, and neoplastic disorders in some individuals.⁶ Increased intestinal permeability has been associated with conditions such as dysbiosis, chronic systemic inflammation, insulin resistance, and metabolic

syndrome. When there are problems in the intestinal barrier and increased intestinal permeability, serum zonulin levels increase.⁷ Elevated zonulin levels have been associated with a greater waist circumference, diastolic blood pressure, fasting glucose, and an increased risk of metabolic disease.⁸

This study aimed to determine how the serum zonulin level is affected in patients in PICU, to evaluate the relationship between anthropometric measurements and biochemical parameters, primarily pre-albumin and Vitamin D, and to determine the utility of the serum zonulin level in the follow-up and management of nutrition.

MATERIAL AND METHOD

The study included 35 critically ill pediatric patients treated in the tertiary-level PICU of Hatay State Hospital. The control group was formed of 25 healthy children who presented at the Pediatric Health and Diseases Polyclinic for routine examinations. For the children in PICU, a record was made of the diagnoses on admission, PIM-3 score, PELOD-2 score, body weight, and height on the first day of admission and on discharge, demographic data, complications that developed during the follow-up period, and the daily calorie intake. The albumin, pre-albumin, Vitamin D, and zonulin levels were examined in patients with findings of infection that regressed during ICU follow-up. Patients with trauma, malignancy, weight >97th percentile, or active infection were excluded from the study. 2. It should be stated how and why it was decided that there was no active infection in excluded patients. Since the children we compared in the control group were healthy children without infection and also because the zonulin level may be affected by the infection, we excluded the patients with active infection. The serum zonulin levels were measured using the Bio-Tek ELX-800 ELISA method in the Health Sciences Vocational Further Education School Research and Application Laboratory of Van Yüzüncü Yıl University. Our study was approved by our hospital's Ethic Committee. All procedures were carried out following the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

A normality test of zonulin values was done with Shapiro-Wilk. The correlation between demographic data, blood parameters, and zonulin was calculated using Spearman's rho. The differences between prealbumin values >20 and <20 and zonulin were analyzed by the Mann-Whitney U Test. Differences between vitamin D values >20 and <20 and zonulin were analyzed by the Mann-Whitney U Test

RESULTS

The 35 patients included in the study were 19 males and 16 females, with a mean age of 48.1 ± 41.8 months and a median length of stay in PICU of 19 (min:13-max:33) days. The reasons for admission to PICU were respiratory failure in 12 patients, central nervous system-related causes in 9 patients, cardiac causes in 8, renal failure in 4, and gastrointestinal bleeding in 2. Blood samples were taken from the patients, and three were fed parenterally and 32 enterally. At the time of admission, the weight percentile was $<3\%$ in 11 (31.4%) patients and in the normal range in 24 (68.6%) (normal range: 3-97%). Throughout ICU follow-up, 14 (40%) patients reached at least 90% of the target calories. The PIM-3 score of the patients was determined to have a mean of 29.8 ± 25.6 and a median of 19 (min:0.5, max: 89). The PELOD-2 score was determined to have a mean of 8.9 ± 4.2 and a median of 9 (min:1, max:18) (Table 1).

The Vitamin D level was determined to have a median of 29 (min: 19- max: 38) ng/mL and <29 ng/mL in 9 (25.7%) patients. The albumin level was determined to have a median of 3.4 (min:3 – max:3.8) mg/dL, and the pre-albumin level have a median of 19 (min:11 – max: 22) mg/dL. The CRP level was determined to have a median of 3 (min:1.6 – max: 5.3) mg/L.

Table 1. Demographic data

		n	% Mean $\pm$ SD
Male		19	%54.1
Weight Percentile	<3	11	%31
	3-10	7	%20
	10-25	9	%26
	25-50	3	%9
	50-75	3	%9
	75-90	1	%3
	90-97	1	%3
Age (Month)		35	$48,1 \pm 41,8$
Length of Stay		35	$24,1 \pm 16,4$
PELOD 2 score		35	$8,7 \pm 4,2$
PIM 3 score		35	$29,8 \pm 25,6$

Table 2. Evaluation of Zonulin levels

	Study Group	Control Group	p
Zonulin	12.8 ± 7.2	6.6 ± 2.3	0.001*

* $p < 0.05$, Evaluated with the Mann Whitney U Test.

The serum zonulin level was found to have a mean of 12.8 ± 7.2 in the critically ill patients and a mean of 6.6 ± 2.3 in the control group. The serum zonulin level in the critically ill patients was statistically significantly higher than in the control group ($p = 0.001$) (Table 2).

There was no statistically significant correlation between zonulin and pre-albumin in critically ill pediatric patients ($r = -0.117$, $p = 0.502$). The difference between the zonulin levels of patients with pre-albumin values <20 and >20 was found to be statistically significant (13.1 ± 6.7 vs. 12.2 ± 8.4 , $p = 0.484$ Mann Whitney U-test). The zonulin – pre-albumin distribution is shown in Figure 1.

A low level significant negative correlation was determined between the zonulin level and Vitamin D level in critically ill pediatric patients (Spearman $r = -0.386$, $p = 0.022$). The zonulin levels of patients with Vitamin D value <20 (16.5 ± 5.4) were found to be statistically significantly higher than the zonulin levels of patients with Vitamin D value >20 (11.5 ± 7.4) $p = 0.033$, Mann Whitney U-test). The zonulin – Vitamin D distribution is shown in Figure 2.

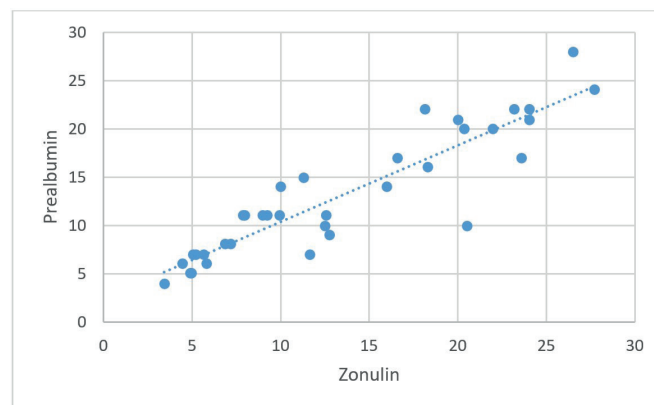


Figure 1. Zonulin – prealbumin distribution chart

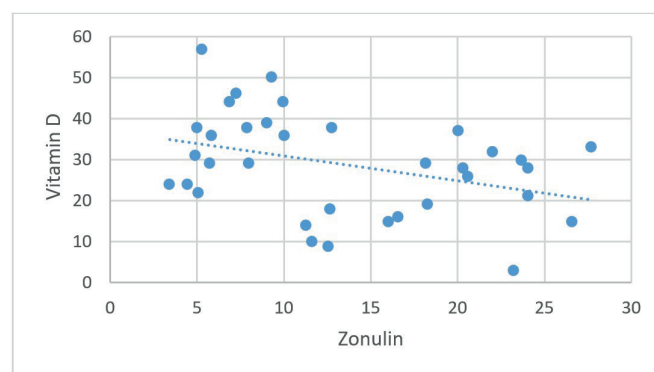


Figure 2. Zonulin – vitamin D distribution chart

No statistically significant correlation was determined between zonulin and creatinine in critically ill pediatric patients (Spearman $r=0.231$; $p=0.182$). No statistically significant correlation was determined between the zonulin value and BUN ($r=0.329$; $p=0.053$), AST ($r=0.251$; $p=0.146$), and ALT ($r=-0.061$; $p=0.726$) levels.

The mean zonulin level was found to be 11.4 ± 7.1 in the patients who reached the target calories and 13.7 ± 7.3 in the patients who did not reach the target calories, and the difference between these two groups was not statistically significant (Mann Whitney U-test, $p=0.429$). The mean Vitamin D level was significantly higher at 36.9 ± 9.8 in patients who reached the target calorie intake compared to 23.4 ± 10.8 in the patients who did not reach the target calories (Independent Paired Samples t-test, $p=0.001$). There was no statistically significant difference in mean pre-albumin levels between the patients who reached and did not reach the target calories (19.4 ± 7.3 vs. 16.8 ± 7.4) (Independent Paired Samples t-test, $p=0.302$). There was no statistically significant difference in mean albumin level between the patients who reached and did not reach the target calories (3.4 ± 0.5 vs. 3.3 ± 0.6) (Independent Paired Samples t-test, $p=0.794$).

DISCUSSION

Zonulin is accepted as a biomarker of increased intestinal permeability. To a significant degree, intestinal permeability is regulated by tight junctions in the intestinal epithelium, and zonulin has an important role in regulating them. The regulation of tight junctions is thought to be responsible for the movement of fluid, macromolecules and leukocytes between the bloodstream and the intestinal lumen.⁸

In a study by Küme et al., zonulin was found to be significantly higher in obese children than in healthy children, which showed the potential role of zonulin in the etiopathogenesis of obesity and related disorders.⁹ In a similar study, energy and carbohydrate intake greater than the daily requirement was shown to play a role in increasing intestinal permeability and increased zonulin levels.¹⁰ In the current study, the zonulin level of critically ill pediatric patients was determined to be higher than that of the healthy control group. The determination of a higher zonulin level in critically ill pediatric patients may be associated with delayed gastric emptying, an abnormal motility pattern, deterioration in the intestinal barrier, prolonged catabolic process, exposure to oxidative and hypoxic stress, and bacterial translocation developing associated with all of these.

Pre-albumin is a plasma protein, a large part of which is synthesized from the liver, which has a half-life approximately two days shorter than that of albumin. Although used in the evaluation of nutritional status in PICUs, it is not accepted as an ideal marker as it is a negative acute phase reactant and production is decreased in inflammation.^{11,12} No significant correlation was determined between zonulin and pre-albumin in the current study. The fact that zonulin was determined to be high in patients with normal pre-albumin levels and low in those with low pre-albumin levels suggests that zonulin cannot be used in the evaluation of acute malnutrition and the monitoring of nutrition.

In 2019, Merker et al. evaluated hospital in-patients and showed a correlation between Vitamin D deficiency and chronic malnutrition.¹³ The results of the current study showed a statistically significant negative correlation between Vitamin D and zonulin. Therefore, the relationship between low Vitamin D and high zonulin levels may be useful in the evaluation of chronic malnutrition.

Serum creatinine levels in children with chronic malnutrition have been previously shown to be lower than in children who are not malnourished.¹⁴ Therefore, considering that the relationship between low creatinine and high zonulin levels may be useful in the evaluation of chronic malnutrition, this relationship was evaluated in this study, but no significant correlation was determined. Finally, since no significant difference was found between the zonulin levels of patients who reached and did not reach the targeted calories, this demonstrated that zonulin would not be a good marker in nutritional surveillance in critically ill pediatric patients.

Limitations of this study are the relatively low number of patients and that basal zonulin levels were unknown. Finally, in our study, the mortality rate in the PICUs was found to be 8.57%. The mortality rates were found to be similar to those in developed countries.

CONCLUSION

This is the first study in the literature to evaluate zonulin as a marker of the nutritional status in critically ill pediatric patients. According to the findings of the study, the serum zonulin level selected as a biomarker for the monitoring and management of nutrition in critically ill pediatric patients is not an appropriate marker. However, this study can be considered valuable in measuring the zonulin level in critically ill pediatric patients for the first time and providing a reference value.

Ethical approval

This study has been approved by the Hatay Mustafa Kemal University Ethics Committee. Written informed consent was obtained from the participants.

Author contribution

Surgical and Medical Practices: AK, YÇ; Concept: AK, GOT, AUK; Design: AK, YA; Data Collection or Processing: SA, TTK; Analysis or Interpretation: YÇ, SA, AUK; Literature Search: YÇ, GOT; Writing: AK. All authors reviewed the results and approved the final version of the article.

Source of funding

The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Feferbaum R, Delgado AF, Zamberlan P, Leone C. Challenges of nutritional assessment in pediatric ICU. *Curr Opin Clin Nutr Metab Care*. 2009;12:245-50. [\[Crossref\]](#)
2. Mehta NM, Skillman HE, Irving SY, et al. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Pediatric Critically Ill Patient: Society of Critical Care Medicine and American Society for Parenteral and Enteral Nutrition. *Pediatr Crit Care Med*. 2017;18:675-715. [\[Crossref\]](#)
3. Alberda C, Gramlich L, Jones N, et al. The relationship between nutritional intake and clinical outcomes in critically ill patients: results of an international multicenter observational study. *Intensive Care Med*. 2009;35:1728-37. [\[Crossref\]](#)
4. Hulst J, Joosten K, Zimmermann L, et al. Malnutrition in critically ill children: from admission to 6 months after discharge. *Clin Nutr*. 2004;23:223-32. [\[Crossref\]](#)
5. Berger MM, Reintam-Blaser A, Calder PC, et al. Monitoring nutrition in the ICU. *Clin Nutr*. 2019;38:584-93. [\[Crossref\]](#)
6. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev*. 2011;91:151-75. [\[Crossref\]](#)
7. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci*. 2000;113 Pt 24:4435-40. [\[Crossref\]](#)
8. Fasano A. Intestinal permeability and its regulation by zonulin: diagnostic and therapeutic implications. *Clin Gastroenterol Hepatol*. 2012;10:1096-100. [\[Crossref\]](#)
9. Küme T, Acar S, Tuhan H, et al. The Relationship between serum zonulin level and clinical and laboratory parameters of childhood obesity. *J Clin Res Pediatr Endocrinol*. 2017;9:31-8. [\[Crossref\]](#)
10. De Santis S, Cavalcanti E, Mastronardi M, Jirillo E, Chieppa M. Nutritional keys for intestinal barrier modulation. *Front Immunol*. 2015;6:612. [\[Crossref\]](#)
11. Pencharz PB. Assessment of protein nutritional status in children. *Pediatr Blood Cancer*. 2008;50:445-6. [\[Crossref\]](#)
12. Shenkin A. Serum prealbumin: is it a marker of nutritional status or of risk of malnutrition? *Clin Chem*. 2006;52:2177-9. [\[Crossref\]](#)
13. Merker M, Amsler A, Pereira R, et al. Vitamin D deficiency is highly prevalent in malnourished inpatients and associated with higher mortality: a prospective cohort study. *Medicine (Baltimore)*. 2019;98:e18113. [\[Crossref\]](#)
14. Hari P, Bagga A, Mahajan P, Lakshmy R. Effect of malnutrition on serum creatinine and cystatin C levels. *Pediatr Nephrol*. 2007;22:1757-61. [\[Crossref\]](#)