Research Article / Özgün Araştırma

DOI: 10.4274/cayd.galenos.2024.19327
J Pediatr Emerg Intensive Care Med

Serum Level of High-mobility Group Box Protein-1 in Children with Sepsis, Severe Sepsis and Septic Shock

Sepsis, Ağır Sepsis ve Septik Şoklu Çocuklarda Yüksek Hareketli Grup Kutu Protein-1'in Serum Düzeyleri

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Abstract

Introduction: Sepsis is an important risk factor for morbidity and mortality in children. Early recognition of sepsis as the most important step in reducing morbidity and mortality. Due to the limitations of current diagnostic tests (i.e., poor sensitivity and delayed results), new research is needed to identify sepsis biomarkers. High mobility group box protein-1 (HMGB1) is one of the late mediators of sepsis. Comparing serum HMGB1 levels between healthy children and those with sepsis is the main goal of our research.

Methods: This prospective multicenter clinical trial. We prospectively collected 43 cases of sepsis treated (3 months to 17 years old) in two different pediatric intensive care units between January 1 and June 30, 2017. The sepsis group was subdivided into sepsis, severe sepsis, and septic shock. The patient and healthy control groups were compared (n=28). The following clinical situations were noted: Pediatric risk of mortality III (PRISM III) and pediatric logistic organ dysfunction (PELOD) scores; need for mechanical ventilation; presence of septic shock; need for plasmapheresis and renal replacement therapy; and death.

Results: Patients with sepsis had significantly increased HMGB1 levels compared with the healthy controls. Serum HMGB1 level was not associated with PELOD and PRISM scores (p>0.05). Serum HMGB1 levels were higher in patients with mortality than in those who survived, but the difference was not statistically significant.

Conclusion: Our study results showed that serum HGMB1 levels were higher in children with sepsis than in healthy children, and HMGB1 levels were also higher in patients with septic shock than in those without shock. More research is required to determine the response to therapy in children with sepsis in the pediatric critical care unit by serially measuring serum HGMB1 levels during the follow-up period.

Keywords: Sepsis, septic shock, high mobility group box protein-1, children, pediatric intensive care unit, mortality risk score

Öz

Giriş: Sepsis, çocuklarda morbidite ve mortalitenin önde gelen nedenlerinden biridir. Sepsisin erken tanınması, morbidite ve mortaliteyi azaltmak için en önemli adımdır. Mevcut tanı testlerinin sınırlamaları nedeniyle (yani zayıf hassasiyet ve gecikmeli sonuçlar), sepsis biyobelirteçlerini tanımlamak için yeni araştırmalara ihtiyaç vardır. Yüksek hareketli grup kutu protein-1 (HMGB1) sepsisin geç mediyatörlerinden biridir.

Yöntemler: Bu ileriye dönük çok merkezli bir klinik çalışmadır. İki farklı çocuk yoğun bakım ünitesinde 1 Ocak-30 Haziran 2017 tarihleri arasında tedavi edilen 43 sepsis olgusu (3 ay-17 yaş arası) ileriye dönük olarak toplanmıştır. Sepsis grubu sepsis hastaları, ağır sepsis olguları ve septik şok olarak alt gruplara ayrılmıştır. Hasta grubu sağlıklı kontrol grubu (n=28) ile karşılaştırılmıştır. Aşağıdaki klinik durumlar not edilmiştir: Pediyatrik mortalite riski III (PRISM III) ve pediyatrik lojistik organ disfonksiyonu (PELOD) skorları; mekanik ventilasyon ihtiyacı; septik şok varlığı; plazmaferez ve renal replasman tedavisi ihtiyacı; ve ölüm.

Bulgular: Sepsisli hastalarda sağlıklı gruba kıyasla HMGB1 anlamlı derecede artmıştır. Serum HMGB1 düzeyleri PELOD ve PRISM skorları ile ilişkili bulunmamıştır (p>0,05). Serum HMGB1 düzeyleri mortalitesi olan hastalarda hayatta kalanlara kıyasla daha yüksekti, ancak istatistiksel olarak anlamlı değildi.

Sonuç: Çalışma sonuçlarımız serum HGMB1 düzeylerinin sepsisli çocuklarda sağlıklı çocuklara kıyasla daha yüksek olduğunu ve HMGB1 düzeylerinin de septik şoklu hastalarda şoksuz sepsis bulgularına kıyasla daha yüksek olduğunu göstermiştir. Sepsisli çocukların pediyatrik yoğun bakım ünitesindeki takipleri sırasında serum HGMB1 düzeylerinin seri olarak değerlendirildiği ve tedaviye yanıtın bir belirteci olarak kullanıldığı ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Sepsis, septik şok, high mobility group box protein-1, çocuk, çocuk yoğun bakım, mortalite risk skoru

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Received/Geliş Tarihi: 25.06.2024 **Accepted/Kabul Tarihi:** 22.08.2024

Introduction

Severe sepsis and septic shock are clinical manifestations of sepsis, which is an infection-related systemic inflammatory response syndrome.[1] The rising prevalence of severe sepsis and septic shock, which affect millions of people annually and kill one in every four (often higher) cases, is an important issue in health care.[2] Sepsis is a potentially fatal organ failure caused by a host's dysfunctional reaction to infection.[3] It is one of the leading causes of admissions to pediatric intensive care unit (PICU). Severe sepsis is defined as an infection with at least one acute organ dysfunction.[4] Sepsis-associated organ dysfunction in children is characterized by severe infection resulting in either non-cardiovascular or cardiovascular organ dysfunction. Septic shock is defined as a severe infection resulting in hypotension, the need for vasoactive medication, or impaired perfusion.[5,6]The management of corticosteroids is complex and requires fluid resuscitation, appropriate administration of antibiotics, vasoactive drugs, and, under specific conditions, corticosteroids. In addition, the use of mechanical ventilators, plasmapheresis, and renal replacement therapy (RRT) can be necessary.[5]

The inflammatory response is the most prominent feature of sepsis. Therefore, studies have been conducted on the host immune response to develop new therapeutic strategies. Pro-inflammatory cytokines [e.g., tumor necrosis factor (TNF)-a and interleukin (IL)-1b] can cause tissue damage, metabolic acidosis, hypotension, multiple organ failure, and even death.[7] Approximately 30 years ago, high-mobility group (HMG) chromosomal proteins were identified in mammalian cells and given the name "HMG" due to their electrophoretic mobility in polyacrylamide gels.[8] The role of HMG1 as a non-histone chromosomal protein in DNA binding and its ability to alter the DNA helical structure was initially recognized in 1978.[9]

High mobility group box protein-1 (HMGB1) is a nuclear protein produced as part of the circulation of oxidative stress by activated macrophages and monocytes. It has pro-inflammatory characteristics. It continues to be evaluated as a potential treatment option because of its prolonged increase of 12-18 hours following TNF-α peaks.[10,11] HMGB1 seems to be a late modulator of life-threatening systemic inflammation in animal models, as well as a participant in delayed endotoxin mortality and systemic inflammation.[12-14] HMGB1 levels were previously studied in critically ill patients with sepsis, severe infection, or acute respiratory distress syndrome.[15,16] However, these studies were limited to a small number of patients in heterogeneous patient groups, especially adults. The purpose of the current study was to assess serum HMGB1 levels in children with sepsis at the time of diagnosis and to further explore its association with scoring systems, laboratory results, and outcomes.

Materials and Methods

Patients admitted to the two different PICUs were evaluated for sepsis prospectively between January 1 and June 30, 2017. Both PICUs are located in two large cities and serve as tertiary care centers for the region. Children aged 3 months to 17 years have been enrolled. Children who were treated due to sepsis at another center were excluded.

Baseline demographic characteristics, including age, sex, and underlying disease (chronic renal dysfunction, heart disease, neurological diseases such as epileptic/genetic syndromes, etc.) have been noted. Laboratory examinations, including hemoglobin, white blood cell count, platelet count, serum C-reactive protein, procalcitonin, fibrinogen, lactate, serum blood urea nitrogen, creatinine, aspartate transaminase, alanine transaminase, and albumin levels, were noted. The pediatric risk of mortality III (PRISM III) and pediatric logistic organ dysfunction (PELOD) scores of all patients were calculated and noted upon admission. Septic shock was defined as persistent hypotension despite adequate fluid resuscitation for at least 1 h. The primary aim of this study was to compare serum HMGB1 levels between children with sepsis and healthy children. The sepsis group was subdivided into sepsis, severe sepsis, and septic shock. Age- and sex-matched healthy children (without chronic and underlying conditions) served as controls. The following clinical situations were noted: Need for mechanical ventilation; Vasoactive amine requirement; Blood product administration; Need for plasmapheresis and RRT; and death. Their relationship with the HMGB1 level was also analyzed.

After the diagnosis of sepsis, which was confirmed by the pediatric intensivist, informed consent was obtained from the parents of the children. Blood cultures and serum samples were collected at the time of sepsis diagnosis. Serum samples were stored at 20 °C until serum HMGB1 analysis. HMGB1 was measured using a commercially available ELISA.

Statistical Analysis

Means and standard deviations were used to summarize quantitative data. Qualitative results were summarized using percentages and quantities. Because of the skewed nature of the data and the small sample size, simple univariate comparisons were performed using non-parametric techniques: Mann-Whitney U rank-sum test was used for quantitative data, and the chi-square test for homogeneity was used for qualitative data. Statistical significance was determined using a two-tailed p-value 0.05.

Results

The present research included 43 children diagnosed with sepsis in two tertiary PICUs using the "Surviving Sepsis

Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock" (1), as well as 28 healthy children as a control group. The demographic and clinical findings and laboratory parameters of children with sepsis are summarized in Tables 1 and 2. The patients were subdivided into three groups: children with sepsis, severe sepsis, and septic shock. A total of 7 patients had an underlying disease (2 patients with congenital heart disease, 5 patients with epilepsy). CRP and procalcitonin levels were significantly higher among patients with sepsis ($p<0.001$) and were significantly higher in the septic shock group compared with the sepsis group ($p<0.001$).

The serum HMGB1 level in the control group was 3.28±0.84 ng/mL. The median HMGB1 concentration of the patient group was 16.3 ng/mL [interquartile range (IQR) 5.3-23.8] and was significantly higher in children with sepsis than in the controls ($p<0.0001$) (Figure 1). When we compared serum HMGB1 levels among children with sepsis, severe sepsis, and septic shock, significantly increased HMGB1 levels were observed in children with septic shock ($p<0.05$ for both) (Figure 2). Serum HMGB1 level was not associated with PELOD and PRISM scores ($p>0.05$). The serum HMGB1 level was 25.7 ng/mL (IQR 4.86-65.5) and higher in the mortality group than in the surviving group (Figure 3).

Twenty-seven (62.7%) of 43 patients with sepsis (cases) required mechanical ventilation (MV). The mean MV time was 6.25±7.8 d. In the case group, the HMGB1 level was

Table 1. Demographic and clinical characteristics of the sepsis and control group

	Control group (n=28)	Sepsis group (n=43)
Age (months)	35.2±47.6 (6-160)	45.9±50.2 (3-200)
Gender (girls/boys)	15/13	22/21
Fever (°C)	36.1±0.6	37.9±1.22
Heart rate (per min.)	92±43	152±23
Respiratory rate (per min.)	26±12	36±19
Capillary refilling time (seconds)	1.5± 0.2	2.8±1.4
PRISM score	0	19.3±11.1
PELOD score	0	16.5±13.5

PRISM: Pediatric risk of mortality, PELOD: Pediatric logistic organ dysfunction

Table 2. Laboratory parameters of sepsis and control group

	Control group (n=28)	Sepsis group (n=43)
Hemoglobin (g/dL)	11.9±3.0	8.9±2.0
WBC (mm^3)	5700 (4800-7600)	11660 (1300-58900)
Platelet count ($10^3/mm^3$)	200 (162-460)	143 (500-789)
Fibrinogen (mg/dL)	260±95	313±167
C-reactive protein (mg/L)	3.1 (2.0-5.0)	59 (17.0-101.2)
Procalcitonin (ng/mL)	0.03 (0-0.04)	12.16 (3-86)
Blood urea nitrogen (mg/dL)	11.9 (5-16)	12.9 (2-73)
Creatinine (mg/dL)	0.32 (0.2-0.6)	0.67 (0.04-2.25)
AST (IU/L)	20 (12-40)	58 (13-4100)
ALT (IU/L)	12 (9-22)	46.5 (6-3450)
Albumin (g/dL)	3.8 (3.6-4.9)	2.78±0.6
Lactate (mmol/L)	0.9 (0.8-1.6)	2.7 (0.7-22)
HMGB1 (ng/mL)	3.28±0.84	16.3 (5.3-23.8)
PaO_2/FiO_2	-	252±92

WBC: White blood cell count, HMGB: High-mobility group box protein-1, PAO_2/FiO_2: Ratio of arterial oxygen partial pressure to fractional inspired oxygen, AST: Aspartate transaminase, ALT: Alanine transaminase

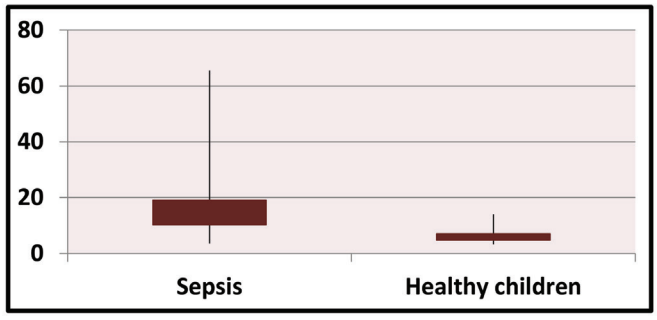

Figure 1. Serum high mobility group box protein-1 levels in sepsis and control groups

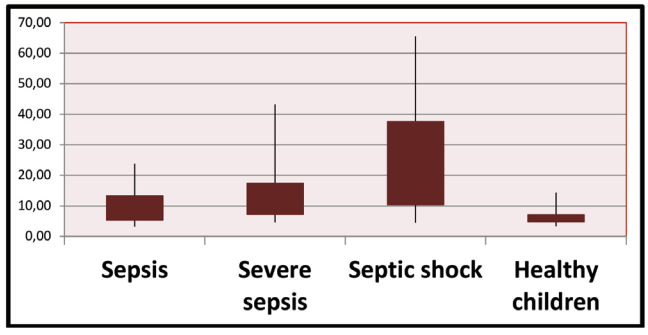

Figure 2. Serum high mobility group box protein-1 levels in children with sepsis, severe sepsis, and septic shock compared with healthy children

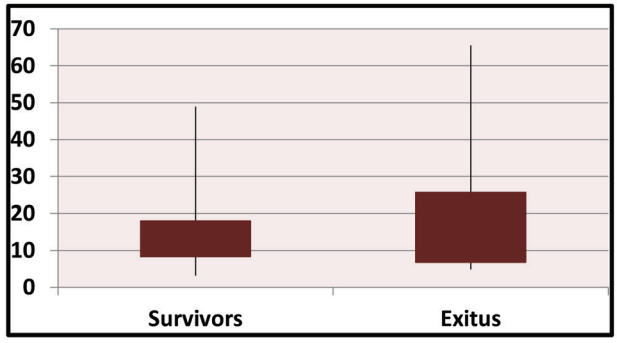

Figure 3. Serum high mobility group box protein-1 levels regarding to prognosis

not associated with the need for MV or the time (d) for MV. Concerning hemodynamic changes. In the patient group, 31 children (72%) required the usage of vasoactive amines. Twenty-eight patients (65.1%) needed a transfusion of bloo1d components. Nine patients (20.9%) received Intravenous immunoglobulin and five patients (11.6%) required corticosteroid treatment. Concerning renal function, the median serum creatinine level was 0.67 (QR 0.04-2.25) mg/dL among the case group. Eighteen patients in the case group (41.8%) had acute kidney injury secondary to sepsis, and eight (18.6%) required renal replacement therapy. Plasmapheresis was performed in six patients (13.9%) with thrombocytopenia associated with multi-organ failure.

The average length of stay of patients in the PICU was 14 days (2-58). Twenty-one (67.4%) were discharged from the PICU before 28 days, six (13.9%) stayed for more than 28 days, and sixteen (37.2%) died.

Discussion

The function of HMGB1 in pediatric disorders needs to be clarified because disease characteristics differ between children and adults. In the present study, we evaluated the relationship between HMGB1 levels and the severity of several pediatric sepsis syndromes and outcomes, such as sepsis, severe sepsis, and septic shock. In all pediatric sepsis situations have higher levels of HMGB1 than healthy participants. There was a significant association between the severity of pediatric sepsis and HMGB1 level. Sepsis is mediated by late-stage HMGB1, as opposed to earlier inflammatory agents like TNF-α and IL-1β. According to the findings of our research, the serum HGMB1 level of children can be a useful marker for both the presence of sepsis and the diagnosis of septic shock.

Clinical observational studies have demonstrated that HMGB1 levels significantly increase in the serum of patients with suspected infection, pneumonia, and sepsis. HMGB1 is typically found at a plasma level of 5 ng/mL in healthy animals and healthy individuals. However, in patients with septicemia, HMGB1 levels are elevated to an average of 25.2 ng/mL in survivors and 83.7 ng/mL in non-survivors.[13] In our present study, HMGBl levels of healthy children were compared, and the result showed that HMGB1 levels of the control group (3.28±0.84 ng/mL) were lower than those of the sepsis group [16.3 ng/mL (IQR 5.3-23.8) ($p<0.001$]. The findings suggested that HMGB1 levels were related to both the development and onset of sepsis. In addition, the serum HMGB1 level of patients in the septic shock group [37.6 ng/mL (IQR 10.4-65.5)] was higher than that of the sepsis group ($p<0.05$).

The role of HMGB1 in the severity and outcome of pediatric sepsis syndromes has not been extensively studied. Serum HMGB1 levels were higher in septic patients who died from infection than in those who survived, indicating that this protein should be further studied as an option for therapy.[14] Sundén-Cullberg et al.[15] observed a rise in serum HMGB1 levels during sepsis. The results revealed that IL-6, IL-8, IL-10, and TNF-α levels were considerably elevated in the shock group. However, contrary to our findings, the authors observed no major difference in HMGB1 levels between the severe sepsis and septic shock patient groups.[15]A different investigation found that the degree of organ failure during septic shock was connected with the plasma concentration of HMGB1 and that the concentration increased over time in patients who were more severely affected.[16] Currently, our understanding of the relationship between local HMGB1 release, serum HMGB1 levels, infection site damage, and organ damage in patients with sepsis remains incomplete.

Similarly, Pavare et al.[17] demonstrated that LPS-binding protein, IL-6, and CRP are correlated with the severity of infections in children, although HMGB1 does not appear to play a significant role. One explanation could be that the investigators looked at early inflammation, whereas HMGB1 is frequently raised in the later phases of sepsis. Our study evaluated HMGB1 levels at the time of sepsis diagnosis without repetitive measurements, but we found a significant difference between the sepsis and septic shock patient groups. Consequently, the impact of HMGB1-induced inflammatory activation in several systems has gained interest. HMGB1 has been highlighted as an alarming sign associated with various disorders connected with inflammation.[18]Although there is currently no efficient treatment for sepsis that results in a high rate of mortality and morbidity, the identification of HMGB1 as a strong late cytokine mediator of endotoxemia and sepsis has opened up new research avenues for the development of therapies for sepsis. Delayed treatment targeting HMGB1 within 24 hours after experimental sepsis is an effective and unique treatment strategy for life-threatening sepsis. Nevertheless, further investigation is needed in other crucial domains, such as the mechanisms governing the release of HMGB1 from cells, surface receptors that engage with HMGB1, and intracellular signal transduction pathways through which HMGB1 acts as a pro-inflammatory cytokine.[18] Examining these issues will enhance our understanding of the role of HMGB1 and potentially provide a way for the development of targeted and time-sensitive treatment medicines that can decrease the severe mortality and morbidity associated with sepsis.

Conclusion

Our study results showed that serum HGMB1 levels were higher in children with sepsis than in healthy children, and HMGB1 levels were also higher in patients with septic shock

than in those without shock. A recent study showed that serum HGMB1 levels are higher in children with multi-organ failure. Our study results also showed a higher proportion of patients who died due to sepsis; however, we did not show statistical significance.

A limitation of our study is the difference in the definitions of sepsis and septic shock in the years when our study was conducted. We also did not evaluate the kinetics of inflammation markers, such as CRP, PCT, and HMGB1 repeatedly during hospitalization. Further studies with serial evaluation of serum HGMB1 levels during the follow-up period of children with sepsis in pediatric intensive care units using as a marker of response to therapy are needed.

Ethics

Ethics Committee Approval: This study was approved by the Eskişehir Osmangazi University Local Ethical Committee (11.01.2016/05).

Informed Consent: After the diagnosis of sepsis, which was confirmed by the pediatric intensivist, informed consent was obtained from the parents of the children

Authorship Contributions

Concept: E.K., D.Y., E.Ç.D., Design: E.K., D.Y., E.Ç.D., Data Collection or Processing: E.K., Ö.Ö.H., F.E., G.B., Analysis or Interpretation: E.K., D.Y., E.Ç.D., Literature Search: E.K., Writing: E.K., E.Ç.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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