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THE ROLE OF HIGH MOBILITY GROUP BOX PROTEIN-1 IN BACTERIAL SEPSIS

BAKTERİYEL SEPSİSTE HIGH MOBILITY GROUP BOX PROTEIN-1'İN ROLÜ

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ABSTRACT

Objective: Sepsis and associated impaired inflammatory response can lead to multi-organ dysfunction and eventually death. HMGB1 is passively secreted by necrotic cells and actively secreted by inflammatory cells and acts as an extracellular signaling molecule. The aim of this study was to compare serum HMGB1 levels between patients diagnosed with sepsis and healthy controls.

Methods: Patients with sepsis who were treated in Gaziantep Medical Park Hospital Intensive Care Unit between 2018-2019 and healthy individuals without any disease who applied to the internal medicine outpatient clinic of the same hospital for routine control were included in the study. HMGB1 levels in patients (just before the start of treatment) and control subjects were quantitatively determined using Bio-Techne kits.

Results: The sample of the study consisted of 71 participants, 36 patients (14 (38.9%) females and 22 (61.1%) males) and 35 healthy volunteers. The mean age of the patients was 70.97±14.72 years (range 43-91). Since the HMGB1 variable did not follow a normal distribution, the difference between the patient and control groups was analyzed using the Mann-Whitney U test, and this difference revealed a statistically significant between-group difference ($p<0.001$). Serum HMGB1 levels were higher in the patient group than in the control group.

Conclusion: In conclusion, a statistically significant difference was observed in serum HMGB1 levels between sepsis patients and healthy controls. HMGB1 may be planned to be used as a diagnostic tool to identify patients with sepsis in future controlled studies with larger samples.

Keywords: Sepsis, bacterial sepsis, High Mobility Group Box 1 protein, HMGB1, inflamation

ÖZ

Amaç: Sepsis ve ilişkili bozulmuş inflamatuar yanıt, çoklu organ disfonksiyonuna ve sonunda ölüme yol açabilir. HMGB1, nekrotik hücreler tarafından pasif olarak salınır ve inflamatuar hücreler tarafından aktif olarak salgılanır ve hücre dışı bir sinyal molekülü görevi görür. Bu çalışmanın amacı, sepsis tanısı alan hastalar ile sağlıklı kontroller arasında serum HMGB1 düzeylerini karşılaştırmaktır.

Yöntem: Çalışmaya Gaziantep Medical Park Hastanesi Yoğun Bakım Ünitesinde 2018-2019 yılları arasında tedavi gören sepsisli hastalar ile aynı hastanenin dahiliye polikliniğine rutin kontrol için başvuran herhangi bir hastalığı olmayan sağlıklı bireyler alındı. Hastalardan (tedavinin başlangıcından hemen önce) ve kontrol deneklerinde HMGB1 seviyeleri Bio-Techne kitleri kullanılarak nicel olarak belirlendi.

Bulgular: Araştırmanın örneklemini 36 hasta (14 (%38,9) kadın ve 22 (%61,1) erkek) ve 35 sağlıklı gönüllü olmak üzere toplam 71 katılımcı oluşturmuştur. Hastaların yaş ortalaması 70,97±14,72 yıl (dağılım 43-91) idi. HMGB1 değişkeni normal bir dağılım izlemediğinden, hasta ve kontrol grupları arasındaki fark Mann-Whitney U testi kullanılarak analiz edildi ve bu fark istatistiksel olarak anlamlı bir grup arası fark ortaya koydu ($p<0,001$). Serum HMGB1 düzeyleri hasta grubunda kontrol grubuna göre daha yüksekti.

Sonuç: Sonuç olarak, sepsis hastaları ile sağlıklı kontroller arasında serum HMGB1 düzeylerinde istatistiksel olarak anlamlı fark gözlendi. HMGB1, daha büyük örneklem içeren gelecekteki kontrollü çalışmalarda sepsis hastalarını tanımlamak için bir tanı aracı olarak kullanılması planlanabilir.

Anahtar Kelimeler: Sepsis, bakteriyel sepsis, High Mobility Group Box 1 protein (HMGB1), enflamasyon

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Introduction

Sepsis is a clinical syndrome caused by a deregulated host inflammatory response to infection accompanied by physiological, biological and biochemical disorders that present with various clinical manifestations. Sepsis and associated impaired inflammatory response may lead to multiple organ dysfunction and eventually death. Over time, there has been a change in the incidence of the organisms causing sepsis. While gram-positive bacteria accounted for the majority of cases of sepsis in the past, septicemia caused by Gram-negative bacteria and fungi has gradually become common due to increased use of immunosuppressive agents.[1,2]

The diagnosis of sepsis is challenging. Most often, treatment is initiated empirically. The definitive diagnosis of sepsis requires a composite of information including clinical, laboratory, radiological and microbiological findings. Early diagnosis of sepsis is crucial to reduce mortality and morbidity and shorten the length of hospital stay.[3] Despite recent advances in the diagnostic tools and therapeutics, sepsis has a current mortality rate of 30%, which clearly underscores the need for more effective ways to diagnose and treat sepsis.[4]

High mobility group box (HMGB) proteins are non-histone nuclear proteins involved in several functions in the cell. While the expression of HMGB3 and HMGB2 is limited to some cells and occurs only during early stages of life, HMGB1 is widely expressed in almost all cells and continues to be expressed in adulthood.[5] HMGB1 is anchored to the nucleus under physiological conditions. In the nucleus, HMGB-1 non-specifically binds to the small groove of DNA and plays a role in the transcription, replication, DNA repair, differentiation and nucleosome formation.[6] In addition to these nuclear functions, HMGB1 is passively released by necrotic cells and actively secreted by inflammatory cells and acts as an extracellular signaling molecule.[7] HMGB1 exerts its extracellular actions through RAGE and TLR4 receptors. HMGB1 has been shown to be involved in sepsis, traumatic shock, autoimmune diseases, fatty liver disease and many cancers.[8,9]

In the present study, serum HMGB1 levels were compared between patients diagnosed with sepsis and healthy control subjects.

Methods

The study enrolled patients with sepsis who were treated at the Intensive Care Unit of Gaziantep Medical Park Hospital between 2018 and 2019 and healthy subjects without any disease who presented to the Internal Medicine outpatient clinic of the same hospital for routine check-up. All participants or their relatives signed written consent before initiation of the study. Approval for the study was obtained from the Ethics Committee for Clinical Trials of SANKO University (10/2018).

The study was conducted in patients with bacteriologically confirmed diagnosis of sepsis based on positive blood, urine and secretion cultures as well as clinical features meeting the SOFA [Sequential (Sepsis-Related) Organ Failure Assessment] criteria. Among healthy subjects, those with a known medical condition (acute infection, diabetes mellitus, hypertension, connective tissue disease, coronary artery disease, cancers excluding prostate cancer, chronic inflammation and autoimmune disease) were excluded.

Approximately 5-6 cc venous blood samples were drawn from the patients (immediately before initiation of treatment) and control subjects under appropriate conditions for routine laboratory work-up and analysis of serum HMGB1 levels. Blood samples were centrifuged for 8-10 minutes at 4000 rpm within 1 hour of collection. Then the samples were taken from -80°C and kept at 4°C in a refrigerator one night before the measurements. After defrosting, the samples were vortexed and added into microplate wells without any delay. The measurements were performed using the ELISA method, with each sample tested in duplicate. Human HMGB1 levels were determined quantitatively using commercially available Bio-Techne kits (Bio-Techne Ltd. Abingdon-Oxfordshire/United Kingdom; catalog number NBP2-62766) in accordance with the manufacturer's instructions. The double-antibody sandwich enzyme immunoassay technique was used for the analysis. All concentration/absorbance curves for human HMGB1 by ELISA tests and related calculations were obtained using the integrated software of the Biotek ELx808 (Winooski, Vermont, USA) absorbance reader.

The test for human HMGB1 had a sensitivity of 8.57 pg/mL and a detection range of 2-2000 pg/mL. Intra-assay and inter-assay coefficients of variation were 8.2% and 10.4% respectively.

Statistical Analysis

All statistical analyses were performed using the SPSS for Windows 15.0 software package. Whether the variables followed a normal distribution was checked using both visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). A p value greater than 0.05 at Kolmogorov-Smirnov test indicated a normal data distribution. In case when the data were normally distributed, the differences between the two groups were assessed using the Student's t-test and if not, Mann-Whitney U test was used to compare the study groups. The overall type 1 error rate was set at 5% for statistical significance. The cut-off values were determined using the Youden Index to maximize the sum of sensitivity and specificity using the sensitivity+specificity-1 equation. Kaplan-Meier estimation was used to assess the survival of the patients. A *p* value less than 0.05 was considered statistically significant.

Results

The study sample consisted of 71 participants in total including 36 patients (14 (38.9%) females and 22 (61.1%)

males) and 35 healthy volunteers. The mean age of the patients was 70.97±14.72 years (range 43-91). Of the patients, 58.3% (n=21) had pneumonia before the onset of sepsis, 27.8% (n=10) had catheter-related sepsis, 8.3% (n=3) had urosepsis and 2.8% had sepsis associated with gastrointestinal and central nervous system infections.

Comorbidities were present in the study patients including atherosclerotic heart disease in 10 (27.8%) patients, cerebrovascular disease in 7 (19.4%) patients, type 2 diabetes mellitus in 8 (22.2%) patients, chronic kidney failure in 6 (16.7%) patients, malignancy in 4 (11.1%) patients and chronic liver disease in one patient. Laboratory findings of the patients on admission are shown in Table 1.

Table 1. Biochemical results of the patients with sepsis and correlation between HMGB1 (r)

Biochemical parameter	Mean ± SD	HMGB1 r
Urea (mg/dl)	103.22±61.53	0.18
Creatinine (mg/dl)	1.87±1.15	0.14
Aspartate transaminase (IU)	60±68.58	-0.04
Alanine transaminase (IU)	44.92±50.78	-0.05
Mean platelet volume	10.67±1.26	0.24
Hemoglobin	9.88±2.01	-0.13
White Blood Cell count	13936.66±9792.53	0.01
Neutrophil count	11517.22±8147.08	0.09
Platelet count (x10^3)	208.52±131.60	-0.29
Lymphocyte count	1055±834.22	-0.4
Albumin (g/dl)	2.67±0.55	0.01
C-reactive protein	179.45±115.68	0.32
Systolic blood pressure (mmHg)	106.58±19.04	0.14
Diastolic blood pressure (mmHg)	58.80±8.35	0.2
Heart rate (/min)	109.22±9.90	-0.06
Respiratory rate (/min)	23.83±3	-0.31
Oxygen saturation (%)	86.63±3.85	0.18
Body temperature (ºC)	36.60±0.72	-0.13
Mean arterial pressure (MAP) (mmHg)	72.75±10.60	0.07
Glasgow Coma Scale (GCS)	8.88±3.43	-0.1
APACHE II score	28.47±8.29	0.22
Alkaline phosphatase (IU)	98.67±52.08	-0.21
Gamma-Glutamyl Transpeptidase (IU)	50.33±34.35	0.05
Sodium (meq/L)	141.86±7.87	0.22
Potassium (meq/L)	4.27±0.86	0.13
Calcium (mg/dl)	8.06±0.87	-0.05
Prothrombin time (sec)	16.16±4.3	-0.11
INR (IU)	1.29±0.38	-0.11
a(PTZ) (sec)	52.91±33.85	0.15
Procalcitonin	12.21±23.48	0.15
Glucose (mg/dl)	178.08±84.99	0.35
Direct Bilirubin	0.41±0.36	-0.03
Indirect Bilirubin	0.34±0.22	0.08
PAO_2/FiO_2	178.25±71.52	-0.16
PO_2	63.61±5.53	0.29
PCO_2	33±7.32	-0.08
PH	7.32±0.06	-0.21
HCO3	20.11±4	-0.25

APACHE: Acute Physiology and Chronic Health Evaluation, SOFA: Sepsis-related Organ Failure Score, r: coefficient of correlation, INR: International Normalized Ratio, a(PTZ): Activated partial thromboplastin time, PO2: Arterial oxygen pressure, PCO2: Arterial carbon dioxide pressure, PAO2/FiO2: Ratio of arterial oxygen partial pressure to fractional inspired oxygen, SD: Standard deviation

When we examined whether biochemical data were correlated with serum levels of HMGB1, a weak positive correlation was detected with serum procalcitonin, C-reactive protein and glucose levels and mean platelet volume.

Since the HMGB1 variable did not follow a normal distribution, the difference between patient and control groups was analyzed using the Mann-Whitney U test which revealed a statistically significant between-group difference (p<0.001). Serum HMGB1levels were higher in the patient group than in control group (Table 2).

Table 2. Comparison of serum HMGB1 levels between patient and control groups

	Patients (n=36)	Controls (n=35)	p
HMGB1 (pg/mL) Median (min-max)	14.39 (5.99-33.01)	9.6 (4.70-18.71)	**<0.001**

n: number of individuals. The Mann-Whitney U test was used for statistical analysis

Among 36 patients with sepsis, 23 died. The mean duration of survival was 23,538±2,479 days (95% CI 18,679-28,397) in patients with sepsis (Figure 1).

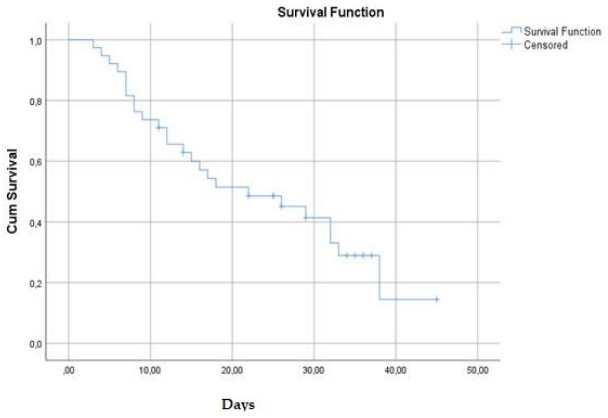

Figure 1. Mean duration of survival in sepsis patients

A significant difference was not found between the patients who died and those who survived after being treated at the ICU with respect to serum HMGB1 concentration (p=0.328). The serum HMGB1 concentration was 15.65 (10.37-29.44) pg/mL in patients who died and 12.60 (5.99-33.01) pg/mL in surviving patients. The following pathogens were isolated from sepsis patients: Escherichia coli (30.6%), Acinetobacter baumanii (25%), Staphylococcus aureus (19.4%) and other (25% in total; 8.3% Klebsiella spp., 8.3% Pseudomonas spp., 2.8% Streptococcus spp., 5.6% Candida spp.).

The association of HMGB1 with the pathogens isolated from cultures of sepsis patients is shown in Table 3. No association was found between serum HMGB1 levels of the patients and any of the pathogens identified from their cultures (p=0.0537).

Table 3. Association between isolated pathogens and serum levels of HMGB1 in patients

	E. coli (n=11)	Acinetobacter baumanii (n=9)	Staphylococcus aureus (n=7)	Other (n=9)	p
HMGB1(pg/mL), Mean±SD	17.49±7.38	15.48±7.35	16.17±3.25	13.39±4.91	0.537

n: number of individuals. SD: Standard deviation. One-way analysis of variance (ANOVA) was used for statistical analysis. Other includes Klebsiella spp., Pseudomonas spp., Streptococcus spp. and Candida spp.).

The cut-off value derived from the ROC curve analysis to distinguish between sepsis patients and control subjects was 12.13 pg/mL (Figure 2).

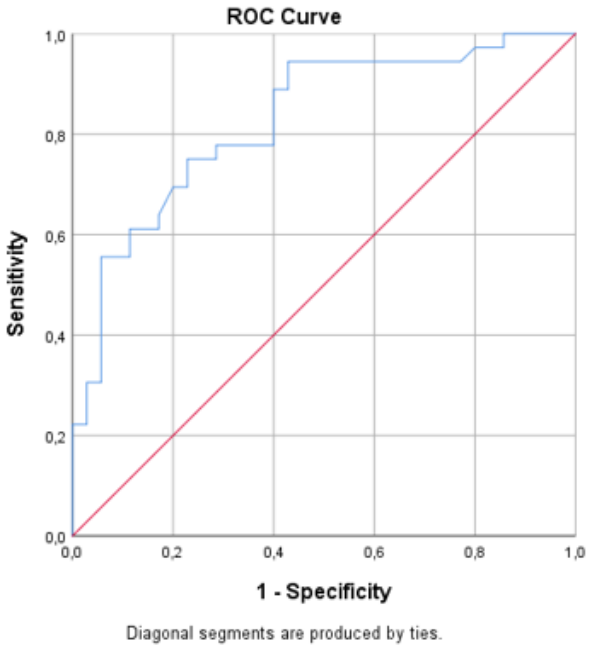

Figure 2. ROC curve analysis between sepsis patients and healthy controls. The area under the curve (AUC) for HMGB1 was 0.827 (95% CI: 0.732-0.923), p<0.001

Discussion

Sepsis is characterized by systemic inflammatory response caused by bacteria and viruses with stimulation of cytokines. Systemic inflammatory response syndrome can also occur as a result of non-infectious conditions such as pancreatitis, burns and tissue injury.[10]

Sepsis is a difficult-to-treat clinical syndrome with a multifactorial etiology.[10] Septic shock, organ failure and death can ensue if adequate treatment is not instituted. In sepsis patients, white blood count differential, blood cultures and serum lactate concentration are initially obtained and this can cause delays in the diagnosis. Additionally, such tests are not reliable to make a diagnosis of sepsis.[11,12] Therefore, novel biomarkers and newer techniques are required for earlier diagnosis of sepsis.[13,16] In the current study, we found higher serum HMGB1 levels in patients diagnosed with sepsis than in control subjects.

HMGB1 is a multifunctional protein involved in inflammation and has a major role in homeostasis. It has been found to contribute to the pathogenesis of sepsis.[17] HMGB1 is actively secreted by immune cells and passively released by necrotic cells. In vitro studies demonstrated that this protein activates inflammation in several types of cell. For example, recombinant HMGB1 was found to induce increased expression of TNF mRNA in human primary blood mononuclear cell cultures (HuPBMCs).[18] Similarly, HMGB1 was shown to promote the release of adhesion molecules (e.g. ICAM-1 and VCAM-1) as well as proinflammatory cytokines such as TNF and IL-8 from microvascular endothelial cells during inflammation and injury.[19]

One study reported that HMGB1 increased local production of proinflammatory cytokines including IL-1β, TNF and MIP-2 (macrophage inflammatory protein-2) in the lung tissue with neutrophil accumulation in lipopolysaccharide-resistant C3H/HeJ mice when administered intrathecally.[20] An in vivo study investigated HMGB1 release in a rat model of endotoxemia or cecal ligation and puncture (CLP). HMGB1 release was first detectable 8 hours after administration of a LD50 dose of endotoxin. After that timepoint HMGB1 increased, reaching a prolonged plateau level from 16 to 32 hours following endotoxin exposure.[21] Similarly, a marked increase was detected in serum levels of HMGB1, beginning 18 hours after surgical induction of peritonitis in a murine sepsis model (CLP).[22]

Clinical studies have shown that serum HMGB1 levels increase significantly in sepsis. In one study, significantly elevated serum levels of HMGB1 were detected in patients with surgical sepsis and sepsis-induced organ dysfunction (hypotension, lactic acidosis, disseminated intravascular coagulation, hypoxemia or decreased urine output) in comparison to healthy subjects.[21]

HMGB1 has been characterized as a late-acting proinflammatory cytokine in the septic process, subsequent to activation of early phase cytokines such as TNF and IL-1.[21,23]

HMGB1 has been identified as a key mediator of organ injury in severe sepsis in humans and experimental animal models.[24] Recent studies found that anti-HMGB1 agents can prevent organ injury.[25,26] Increased local and systemic HMGB1 levels were demonstrated in a study in gnotobiotic piglets infected with enteric pathogens.[27]

In the present study, higher serum HMGB1 levels were detected in sepsis patients than in control subjects (p<0.001). There was no significant difference in serum HMGB1 between sepsis patients who died at the ICU and those who survived (p=0.328). In a 1999 study, Wang H et al. found significantly higher serum levels of HMGB1 in mice who did not survive as compared with survivors in a murine model of sepsis.[21]

In our study, serum HMGB1 levels were not associated with the pathogens isolated from blood cultures in sepsis patients (p=0.0537). Serum HMGB1 levels were 17.49± 7.38 pg/mL in sepsis patients with blood cultures positive for E. coli growth (n=11), 15.48±7.35 pg/mL in patients with Acinetobacter baumanii (n=9), 16.17±3.25 pg/mL in patients with Staphylococcus aureus (n=7) and 13.39±4.91 pg/mL in patients with positive cultures for other infectious agents (n=9) (Klebsiella spp.,

Pseudomonas spp., Streptococcus spp. and Candida spp.) respectively.

Contrastingly, in a study involving sepsis patients, Gaïni S et al. detected elevated serum levels of HMGB1 among bacteraemic patients than in non-bacteraemic patients.[28] In conclusion, a statistically significant difference was observed in serum HMGB1 levels between sepsis patients and healthy controls. The originality of the present study lies in the fact that it was the first study to compare serum HMGB1 levels in relation to infectious agents in sepsis patients. HMGB1 may be established as a diagnostic tool to identify sepsis patients in future controlled studies involving larger sample sizes.

Ethical Approval

All participants or their relatives signed written consent before initiation of the study. Approval for the study was obtained from the Ethics Committee for Clinical Trials of SANKO University (10/2018).

Conflicts of Interests

The authors declare that they have no competing interests.

Author Contribution

All authors contributed equally to this work.

Financial Disclosure

The authors declared that this study has received no financial support.

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