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Diagnostic Value of HMGB1 Protein Levels in Colorectal and Gastric Cancer

Kolorektal ve Gastrik Kanserde HMGB1 Protein Düzeylerinin Tanısal Değeri

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Abstract

Background: High Mobility Group Box-1 (HMGB1), a nuclear protein, regulates the transcription of multiple genes. The study aimed to ascertain the diagnostic utility of HMGB1 protein levels in colorectal and gastric cancers.

Materials and Methods: This was a cross-sectional study. The patients were categorized into groups based on their histopathological diagnoses. Preoperative and pre-chemotherapy blood samples were collected from the patients. The analysis procedures were replicated twice for each sample utilizing the sandwich enzyme immunoassay technique.

Results: The study included 20 controls and 70 patients in total. Of the 70 patients, 24 had a gastric cancer diagnosis, and 46 had a colorectal cancer diagnosis. The patient group did not differ in any of the following categories from the control group: gender, age, body mass index (BMI), alcohol consumption, or smoking. ($p > 0.005$) Pathologic diagnoses did not differ between adenocarcinoma and signet ring cells in gender, BMI, HMGB1 serum levels, smoking, or alcohol consumption ($p>0.005$). The High Mobility Group Box-1 variable was not statistically significant between colorectal and gastric cancer groups in adenocarcinoma patients, who were excluded from the pathology results of 7 signet ring cell carcinoma patients ($p=0.885$).

Conclusions: In conclusion, HMGB-1 protein levels were not different in patients with colorectal and gastric carcinoma compared to those in healthy subjects. HMGB-1 level measurement may not make a diagnostic difference in the presence of colorectal carcinoma and gastric carcinoma.

Keywords: High Mobility Group Box-1, colorectal cancer, gastric cancer, signet ring cell carcinoma

ÖZ

Amaç: Nükleer bir protein olan Yüksek Mobilite Grup Kutusu-1 (HMGB1), birden fazla genin transkripsiyonunu düzenler. Çalışmanın amacı, kolorektal ve gastrik kanserlerde HMGB1 protein düzeylerinin tanısal faydasını belirlemektir.

Gereç ve Yöntem: Bu kesitsel çalışmada hastalar histopatolojik tanılarına göre gruplara ayrıldı. Hastalardan ameliyat öncesi ve kemoterapi öncesi kan örnekleri toplandı. Analiz prosedürleri her örnek için sandviç enzim immünoassay tekniği kullanılarak iki kez tekrarlandı.

Bulgular: Çalışmaya toplam 20 kontrol ve 70 hasta dahil edildi. 70 hastanın 24'ünde mide kanseri tanısı, 46'sında ise kolorektal kanser tanısı vardı. Hasta grubu, cinsiyet, yaş, vücut kitle indeksi (VKİ), alkol tüketimi ve sigara kullanımı kategorilerinden hiçbirinde kontrol grubundan farklı değildi. ($p > 0,005$) Patolojik tanılar, adenokarsinom ve signet halka hücreleri arasında cinsiyet, VKİ, HMGB-1 serum düzeyleri, sigara kullanımı veya alkol tüketimi açısından farklılık göstermedi ($p>0,005$). HMGB-1 değişkeni, 7 signet halka hücreli karsinom hastasının patoloji sonuçlarından hariç tutulan adenokarsinom hastalarında kolorektal ve gastrik kanser grupları arasında istatistiksel olarak anlamlı değildi ($p=0,885$).

Sonuç: Sonuç olarak, kolorektal ve gastrik karsinomlu hastalarda HMGB-1 protein düzeyleri sağlıklı bireylere göre farklı bulunmamıştır. HMGB-1 düzeyi ölçümü kolorektal karsinom ve gastrik karsinom varlığında tanısal bir fark yaratmayabilir.

Anahtar kelimeler: Yüksek Mobilite Grubu Kutu-1, kolorektal kanser, mide kanseri, yüzük hücreli karsinom

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Highlights

- High Mobility Group Box-1 (HMGB1), a nuclear protein, regulates the transcription of multiple genes.
- HMGB-1 protein levels were not different in patients with colorectal and gastric carcinoma compared to those in healthy subjects.
- HMGB-1 level measurement may not make a diagnostic difference in the presence of colorectal carcinoma and gastric carcinoma.

Introduction

Colorectal and gastric cancers are gastrointestinal cancers with high mortality rates. Gastric cancer has remained the fifth most common type of cancer worldwide, despite a steady decline over the last 50 years (1). By 2020, more than one million people worldwide had been diagnosed with gastric cancer, accounting for 5.6% of all cancer cases. While gastric cancer was the leading cause of cancer-related deaths until the mid-1990s, it is now the third leading cause of cancer-related deaths (2). Colorectal cancer (CRC) is steadily increasing worldwide. Given demographic projections, the number of CRC cases worldwide is expected to increase by 60% by 2030, reaching more than 2.2 million new cases and approximately 1.1 million deaths annually. Currently, Eastern Europe, Asia and South America are experiencing rapid increases in both CRC incidence and mortality (3). Although effective therapeutic strategies for CRC have been developed over the past decades, five-year overall survival rates remain poor. This is due to the presence of poor prognostic factors such as vascular and neural invasion, low lymphocyte-monocyte ratio (LMR), late diagnosis and tumor stage. According to estimates, approximately 20% of CRC patients have already progressed to metastatic status at the time of diagnosis (4,5). In addition to surgery, chemotherapy and radiotherapy, new methods such as immunotherapy have recently been used in the treatment of these cancers. The poor prognosis despite these treatments has led to the search for new prognostic and predictive markers. Identification of new targets in treatment is also gaining importance. HMGB1, a high-mobility group box-1 protein, is known to be a nuclear protein that resides in the nucleus and controls the transcription of multiple genes (6). This protein is composed of 215 amino acids and contains two DNA-binding domains, Box-A and Box-B, as well as a C-terminal region that is negatively charged. Recent studies have shown that HMGB1 serves not only as a core protein, but also as a separate cytokine that enhances the production of proinflammatory cytokines. HMGB1 is released from necrotic cells through passive leakage, but it is actively released from inflammatory cells and has a strong tendency to bind to certain receptors (7). HMGB1 serves as an external signaling molecule in processes such as inflammation, cell differentiation, cell migration, and tumor metastasis. It has been observed that HMGB1 protein and the receptor for advanced glycation end products (RAGE), which are crucial for the growth of tumor cells, have been identified as a ligand-receptor pair (8). HMGB1 has been associated with several different types of cancer. Specified cancers include melanoma, prostate, pancreatic and breast cancer (9). High expression of HMGB1 in many of these cancers is often associated with invasion and metastasis. This suggests that HMGB1 plays an important role in cell growth, invasion and cancer metastasis (10). This study was conducted to investigate the expression status and predictive value of HMGB1 in primary colorectal and gastric cancer.

Material and Methods

Study Design and Setting: This study was conducted in the context of a cross-sectional study. The study included patients with histopathologically confirmed primary gastric and colorectal cancer, who were diagnosed at SANKO University Medical Oncology Clinics and had not yet received treatment. Additionally, healthy volunteers, who applied to the SANKO University Check-up outpatient clinic and agreed to participate in the study, were also included. Patients, who had prior exposure to chemotherapy or radiotherapy, were below 18 years old, had undergone surgery, had a previous malignancy, declined to participate in the study, were pregnant, had any autoimmune disease, collagen tissue disease, or severe psychological disorder, and had a history of polypharmacy were not included in the study.

The volunteers were excluded if they had undergone chemotherapy or radiotherapy, surgical intervention, had a history of another malignancy, refused to participate in the study, were pregnant, had any autoimmune disease, collagen tissue disease, severe psychological disorder, or a history of polypharmacy.

The study involved a review of the patients' files to gather information such as age, gender, and routine laboratory test results. The patients were categorized into groups based on their histopathological diagnoses. Pre-

treatment blood samples were collected prior to initiating first-line systemic chemotherapy.
Each volunteer in the study provided approximately 4-5 cc of venous blood, which was collected in a biochemistry tube. The blood was then kept at room temperature for 20 minutes before being centrifuged at 4000 rpm for 10 minutes. This process resulted in the separation of the serum portion. The serum that had been separated was allocated into labeled eppendorf tubes and kept in the deep freezer at -80 ºC in the SANKO University laboratory until the day of the study. The control group underwent the same method of study, using identical parameters. The HMGB1 serum levels were quantified using Rel Assay Brand commercial kits in the designated laboratory, following the instructions provided by the manufacturer. The analysis procedures were replicated twice for each sample utilizing the sandwich enzyme immunoassay technique.
The assay's concentration/absorption graphic curves of the test and calculations regarding the results were performed by using 450 nm wavelenght on the program of the Biotek_ELx808 (Winooski, Vermont, USA) device. The test was determined to have a sensitivity of 0.09 ng / mL and detection range of 1-32 ng / mL. Intra-assay and inter-assay variation coefficients were determined as 8.7% and 5.3% respectively

Ethics: This Study approval was obtained from the SANKO University, Clinical Research Ethics Committee (Approval No. 2022/21.01. date: 22.12.2022). Data from patients who gave informed consent for the use of their information and permission for contact were included in the study. We ensured the patient's confidentiality and followed the principles of the Declaration of Helsinki.

Statistical analyses
To analyze the variables, we employed the statistical software SPSS 26.0, developed by IBM Corporation, headquartered in Armonk, New York, United States.The Shapiro-Wilk-Francia test was used to determine whether or not the data followed a normal distribution, and the Levene's test was used to determine whether or not the fluctuations in the data were consistent with one another. A T test with independent samples and Bootstrap results, as well as a Mann-Whitney U test with Monte Carlo results, were utilized in order to compare two independent groups in terms of quantitative variables. The Pearson Chi-Square Fisher test was utilized for the purpose of comparing categorical variables. The Monte Carlo simulation method was utilized in order to test the exactness of the tests. In the tables, quantitative variables were presented in the form of mean (standard deviation) and median (minimum or maximum), whereas categorical variables were presented in the form of n (percent). A level of confidence of 95% was used to conduct the analysis of the variables, and a p-value of less than 0.05 was regarded as statistically significant.

Results:
A total of 70 patients and 20 controls participated in the study. 24 of the 70 patients were diagnosed with gastric carcinoma, and 46 were diagnosed with colorectal carcinoma. When compared to the control group, the patient group did not differ from the control group in terms of gender, age, body mass index (BMI), smoking, or alcohol consumption. ($p > 0.005$) Additionally, there was no difference in the levels of HMGB1 serum found between the control group and the carcinoma patient group ($p > 0.005$). In terms of gender, age, HMGB1 serum levels, smoking, and alcohol consumption, there was no difference between patients with colorectal carcinoma and patients with gastric carcinoma when the patient group was analyzed in detail ($p > 0.005$). Between the two groups, the only thing that was found to be different was the BMI. In the group that had colorectal carcinoma, the body mass index (BMI) was statistically lower ($p = 0.011$). In terms of pathologic diagnoses, there was no difference between the groups of adenocarcinoma and signet ring cells in terms of gender, body mass index (BMI), HMGB1 serum levels, smoking, or alcohol consumption ($p>0.005$). The only individuals who had a younger mean age were those, who had signet ring cell carcinoma (55.9 years compared to 44.9 years $p = 0.001$). A comprehensive presentation of the demographic and clinicopathologic data pertaining to the groups can be found in Table 1. The comparison of the High Mobility Group Box-1 variable between colorectal and gastric cancer groups in the adenocarcinoma patients, who were excluded from the pathology results of 7 patients with signet ring cell carcinoma, did not show any statistical significance ($p=0.885$).

Table 1: Cancer Types, Gender, and Age Distribution of Patients

	Gender		Age, years	BMI, kg/m²	HMGB-1	Smoke		Alcohol	
	Female, (%)	Male, (%)				No, (%)	Yes, (%)	No, (%)	Yes, (%)
Groups									
Control (n=20)	10 (50.0)	10 (50.0)	54.7± 11.8	25.2± (1.8)	22.6 (13.2-74.2)	9 (45.0)	11 (55.0)	12 (60.0)	8 (40.0)
Patient (n=70)	31 (44.3)	39(55.7)	58.4 ± 11.0	25.3 ± (1.4)	18.3 (8.3 -118.4)	25 (35.7)	45 (64.3)	51 (72.9)	19 (27.1)
p	0.800 [c]		0.236 [t]	0.879 [t]	0.139 [u]	0.602 [c]		0.407 [c]	
Type of CA									
Colon (n=46)	18 (39.1)	28(60.9)	61 (38-80)	24.8 (22-27.7)	18.8 (11.1 - 118.4)	14 (30.4)	32 (69.6)	33 (71.7)	13 (28.3)
Gastric (n=24)	13 (54.2)	11(45.8)	59 (27-67)	25.4 (24.1- 29.1)	18.3 (8.3 -117.7)	11 (45.8)	13 (54.2)	18(75.0)	6(25.0)
p	0.312 [c]		0.194 [u]	0.011 [u]	0.600 [u]	0.293 [c]		0.788 [c]	
Pathology result									
Adenocarcinoma (n=63)	27 (42.9)	36(57.1)	59.9± 9.8	25.3± 1.4	18.3 (8.3-118.4)	21 (33.3)	42 (66.7)	46 (73.0)	17 (27.0)
Stone ring cell (n=7)	4 (57.1)	3 (42.9)	44.9 ± 12.2	25.1 ± 0.4	16.5 (13.2-32.7)	4 (57.1)	3 (42.9)	5 (71.4)	2 (28.6)
p	0.692 [f]		0.001 [t]	0.342 [t]	0.419 [u]	0.239 [f]		0.999 [f]	
Total (n=90)	41 (45.6)	49(54.4)	57.5 ± 11.2	25.2± 1.5	19.7 (8.3-118.4)	34 (37.8)	56 (62.2)	63 (70.0)	27 (30.0)

Abbreviations: [t] *Independent Samples T Test (Bootstrap),* [u] *Mann Whitney U Test (Monte Carlo),* [c] *Pearson Chi Square Test (Monte Carlo),* [f] *Fisher Exact Test(Monte Carlo), HMGB-1: (High Mobility Group Box B-1)*

Discussion

HMGB-1 is a protein with intracellular and extracellular functions. It is structurally defined as a nucleoprotein that binds to DNA and plays an important role in the regulation of gene expression. HMGB-1 acts as an active mediator in the processes of inflammation, tissue repair and cell death. When released into the extracellular environment, it can act as a proinflammatory cytokine and trigger various cellular responses (9-10). In CRC related research, increased levels of HMGB-1 have been observed to have significant effects on the course and prognosis of CRC disease. Patients with high HMGB-1 expression have been found to have more advanced TNM stages of disease, shorter overall survival and disease-free survival, and a higher likelihood of disease recurrence and metastasis (11). It has also been observed in studies that increased expression of HMGB-1 may be associated with damage to the intestinal mucosal physical barrier and alteration of the local immune barrier (12). HMGB-1 is thus considered an independent risk factor for death and relapse risk in CRC patients and a potential marker for a better understanding of the diagnosis and prognosis of CRC. However, definitive data are still lacking in the literature (13). Although some studies have shown some associations of HMGB-1 protein with CRC, it has not been reported to be associated with certain clinical features and to have any diagnostic utility. In particular, HMGB-1 was not associated with gender, age, tumor diameter and nerve invasion (14). In our study, there was no difference in protein levels between the patient population and controls. A recent study also revealed that high levels of HMGB1 have been observed in exosomes derived from gastric cancer cells, which may contribute to cancer progression by inducing M2-like macrophage polarization (15). Therefore, the document focuses on data showing a negative association between HMGB1 and gastric cancer. Studies have shown that HMGB-1 protein plays an important role in the growth, invasion and spread of gastric cancer and is also involved in mechanisms that promote cancer cell survival. HMGB1 has been shown to affect the growth and invasion of gastric cancer cells through the NF-kB pathway and also to promote autophagy-mediated survival of gastric cancer cells through RAGE activation. Furthermore, it is suggested that targeting HMGB1 and its related pathways may be a potential strategy for the treatment of gastric cancer (16-17). These data suggest that HMGB-1 protein is an important factor in the pathogenesis of gastric cancer and can be considered as both a prognostic indicator and a potential therapeutic target. However, in our study, no correlation was found between gastric cancer and HMGB-1 levels. When we evaluate these two results together, although a prognostic relationship with HMGB-1 levels has been reported in the literature, according to the results of our study, no relationship was found with the presence of gastric or colorectal carcinoma. The limitations of our study

include the lack of subgroups according to tumor staging, the lack of concomitant gene expression levels while working at the protein level, and the lack of specific protein determinations such as western blot at the tissue level. Even after excluding the signet ring cell carcinoma subgroup, which is a pathologic subtype with a poor prognosis, still no significant difference was observed in HMGB-1 levels. Another limitation of our study is that the sample was small in number, consisting of patients diagnosed at a single center and volunteers. It is important to remember that when the sample is small, it will be difficult to find significant correlations in the data. Repeating the study with a larger sample will contribute to the literature.

Study limitations

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Conclusion

HMGB-1 protein levels were not different in patients with colorectal and gastric carcinoma compared to those in healthy subjects. HMGB-1 level measurement may not make a diagnostic difference in the presence of colorectal carcinoma and gastric carcinoma.

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Ethical Approval: *This Study approval was obtained from the SANKO University, Clinical Research Ethics Committee (Approval No. 2022/21.01. date: 22.12.2022). Informed consent was obtained from all patients*
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