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Research Article

Investigation of High-Mobility Group Box 1 Protein Levels in Gastrointestinal Malignities

Hanım Seval Savaş[1], Hülya Çiçek[2]*, Mustafa Yildirım[3] and Hüseyin Gürbüz[4]

[1]Specialist Dietitian, Department of Medical Biochemistry, Gaziantep University Institute of Health Sciences, Gaziantep, Turkey

[2]Professor, Department of Medical Biochemistry, Gaziantep University School of Medicine, Gaziantep, Turkey

[3]Professor, Faculty of Medicine, Department of Internal Medicine and Medical Oncology, SANKO University, Gaziantep, Turkey

[4]Gaziantep Dr. Ersin Arslan Training and Research Hospital, Department of Emergency Medicine, Gaziantep, Turkey

***Corresponding Author:** Hülya Çiçek, Professor, Department of Medical Biochemistry, Gaziantep University School of Medicine, Gaziantep, Turkey.

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Abstract

Cancer is a serious health problem that is common in humans and its development is quite complex. Protein-coding genes, tumor suppressor genes, and some biomarkers responsible for the growth, differentiation, and metastasis of cancer cells are used in the diagnosis and treatment of cancer patients. Gastrointestinal cancers are the most common cancers worldwide and cause more deaths than any other type of cancer. The poor prognosis of patients diagnosed with gastrointestinal cancer has made primary prevention a potentially attractive approach. Despite improved diagnosis and treatment methods, survival rates are low. Therefore, early diagnosis and treatment of cancer are important. High Mobility Group Box 1 (HMGB1), an important biomarker in cancers, regulates DNA and transcription. HMGB1 plays a role in cancer progression, angiogenesis, and metastasis development.

Plasma samples were used in this case-control study involving 68 gastrointestinal cancer patients and 40 healthy controls. HMGB1 was measured by the ELISA method.

There was a significant relationship between the patient and control groups in terms of HMGB1 level. It was determined that 98.5% sensitivity, 90% specificity, and 19.31 values could distinguish the patient and control groups. There is no significant difference in HMGB1 levels among gastrointestinal malignancy groups. We found evidence of a significant association between HMGB1 protein and all gastrointestinal cancers. Since results suggest that HMGB1 may play an important role in cancer diagnosis, studies on this marker should be increased.

HMGB1 may be a new serological biomarker for its contribution to early diagnosis and treatment in cancers with poor prognosis and high mortality, such as the digestive system.

Keywords: HMGB1; Gastrointestinal Cancer; Cancer Diagnosis

Introduction

HMG (High Mobility Group) is a set of chromatin proteins [1]. HMGB, which belongs to the HMG protein family, is much more dynamic than other types. The HMGB protein has 3 types of members, HMGB1, HMGB2, and HMGB3. The three proteins are more than 80% identical at the amino acid level and their

biochemical properties are indistinguishable [2]. The high mobility group box protein 1 (HMGB1) consists of 215 amino acids organized into two HMG boxes with DNA binding domains and an acidic C-terminal [3]. HMGB1 plays a role in the regulation of transcription by stably binding to the minor groove of DNA in the nuclei of cells [3,4]. In addition, they regulate the expression of genes in normal or pathological conditions [1]. HMGB1 interaction with DNA is regulated by some transcription factors, including p53, steroid hormone receptors. HMGB1 is secreted actively from necrotic cells and inactive from inflammatory cells, and binds to its most important receptor, RAGE [Receptor for advanced glycation end-products] [5]. Besides HMGB1 RAGE [Toll-like receptors] bind to many receptors such as TLR-2, TLR-4, TLR-9 [6]. In this way, extracellular signal-regulated kinase induces canonical nuclear factor-kB (NF- kB) dependent transcription through its phosphorylation [7,8]. HMGB1's cytokine stimulating effects, binding to receptors, and extracellular signaling cause it to play a role in cell differentiation, cell migration, tumor metastasis, and inflammation [5]. The release of HMGB1 greatly reduces a cell's ability to promote inflammation, proving that it can signal its death to its neighbors. Thus, cells undergoing apoptosis are programmed to hide the signal emitted by cells damaged or killed by trauma [9].

HMGB1 is a highly conserved chromosomal protein that functions as a DNA chaperone inside the cell. Outside the cell, it is a prototype injury-associated molecular model that acts with cytokines, chemokines, and growth factors [10]. Extracellularly released HMGB1 is effective in many types of cancer, regardless of cancer type, including gastric cancer, colorectal cancer, pancreatic cancer, and esophageal cancer [11,12].

Loss of HMGB1 in the pancreas is associated with chromosomal instability characterized by oxidative DNA damage, chromosomal rearrangements, and telomere abnormalities. These lead to inflammatory nucleosome release [13]. In gastric cancer cells, HMGB1 is released following chemotherapy-induced protective autophagy in cells as a damage-related molecular model [14]. Activation of the NF-kB pathway via HMGB1 facilitates colorectal cancer (CRC) cell invasion [15]. Evidence is presented that HMGB1, a chromosomal protein, plays a role in the regulation of the Hippo pathway during liver tumorigenesis [16]. Moreover, HMGB1 shows a unique perioperative inflammatory state in patients with liver cancer. Serum HMGB1 can act as a marker to monitor the surgical course in patients undergoing surgery for liver cancer [17].

Studies like these suggest that HMGB1 is at the center of many of the findings in cancer and normal wound healing [6]. HMGB1 and its receptor RAGE have become an important therapeutic tool. For this reason, it is important to inform the role of HMGB1 in predicting treatment and determining the course of the disease, and in showing which cancers can be targeted in studies [18].

In this study, it was aimed to investigate the HMGB1 levels, which we predict to change in your blood samples, in malignant tumors in the digestive system, in order to detect the disorders occurring in patients with cancer in the digestive system organs such as stomach, intestine, and esophagus and to bring new approaches to cancer diseases.

Material and Methods

Patients who were followed up with a histopathologically confirmed diagnosis of gastrointestinal cancer in the Medical Oncology Clinic of Medicalpark Gaziantep Hospital between 2019-2020 were included in the study. This is a prospective case control study that 68 patients diagnosed with gastrointestinal cancer and 40 control groups were included. Patient files were scanned and information such as age, gender, routine laboratory tests, smoking, and alcohol use was obtained. This study was approved by Gaziantep University Clinical Research Ethics Committee and all participants were given written informed consent. All cancer patients were diagnosed for the first time during enrollment and blood samples were taken before starting systemic drug therapy. Blood samples taken from patients diagnosed with gastrointestinal cancer were grouped according to the type of cancer (stomach, rectum, bile, liver, colon, pancreas, and anal). Our patient and control samples were taken from people who applied to Medical Park Hospital and collected in same place in favorable conditions. Experimental studies were carried out using equipment in Gaziantep University Faculty of Medicine, Department of Medical Biochemistry.

Measurement of serum HMGB1 levels

After sterilization by wiping the forearm antecubital area with 70% alcohol cotton from 68 patients diagnosed with gastrointestinal cancer and 40 age-sex-matched healthy volunteers, 1 tube (5 ml) of blood was collected for routine analysis. The blood samples taken for the study were kept at room temperature for 20 minutes, then the blood samples were centrifuged at 4000 rpm for 10 minutes and the serum part was separated. The separated serum was

divided into portions into labeled Eppendorf tubes and stored at -80 ºC until the study day. When the study will be conducted, the samples were transferred from Gaziantep Medicalpark Hospital to Gaziantep University Basic Medical Sciences Medical Biochemistry Department Laboratory, following the cold chain. HMGB1 was assayed with the commercially available HMGB1 Bioassay ELISA (Enzyme-Linked ImmunoSorbent Assay) kit.

HMGB1 levels were determined manually with the ELISA kit, following the manufacturer's instructions (Bioassay Technology Laboratory, China). The minimum detectable level of human HMGB1 was 0.06 ng/ml and the standard curve range was 0.5-150 ng/ml. The intra and inter-assay coefficients of the variations are <8% and <10%, respectively. Results were calculated using a calibration curve prepared from standards.

Statistical analysis

Statistical analyzes were performed using SPSS for Windows 15.0 software. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/ Shapiro-Wilk tests). Conditions with a p-value above 0.05 in the Kolmogorov-Smirnov test were accepted as a normal distribution. Since the HMGB1 value measured in the patient and control groups did not show normal distribution, the patient and control groups were compared using the Mann - Whitney U test. The Kruskal -Wallis test was used to determine whether GIS malignancies differed in terms of HMGB1 level. The differences between the patient and control groups were evaluated with the Chi-square test. Diagnostic decision-making properties of serum HMGB1 levels in determining the patient and control groups were analyzed by Receiver Operating Characteristics (ROC) curve analysis. In the evaluation of the area under the curve, the cases where the Type 1 error level was below 5% were interpreted as the diagnostic value of the test was statistically significant. With Kaplan Meier survival analysis, the relationship between each of the conditions with HMGB1 below and above the mean and survival was analyzed. Statistical differences were confirmed by the Log-rank test.

Results

Characteristics of the patients

The studied group consists of 108 subjects, 40 controls, and 68 patients. The mean age of the 68 patient groups, 41 (56.5%) men and 27 (43.5%) women, was 60.8 ± 12.6 (range 29-85). The laboratory values of the patients are explained in table 1.

	Patient (n = 68)	**Patient (n = 68)**
	Mean, Standard Deviation	**Median**
Age	60.8 ± 12.6	62
BUN (mg/dl)	15.3 ± 6.5	14
CRE (mg/dl)	0.87 ± 0.27	0.84
AST (U/L)	19.5 ± 9.9	18
ALT (U/L)	26 ± 24.1	16
LDH (U/L)	171.6 ± 44.2	162.5
Albumin	3.8 ± 0.41	3.9
WBC (10^3/mm^3)	9.08 ± 3.36	8.67
Neutrophil	5.78 ± 3.08	4.99
Lymphocyte	2.22 ± 1.13	1.93
PLT (10^3/mm^3)	333 ± 134	304
Hb (g/dl)	12.5 ± 1.7	12.5
MCV (fl)	84.9 ± 7.81	86.1

Table 1: Mean age of patients and levels of blood parameters.

Considering the distribution of 68 patients in terms of gastrointestinal cancer types: 20 include colon, 18 stomachs, 11 recta, 7 liver, 5 gallbladder, and biliary tract, 5 pancreatic and 2 anal cancers. Considering the types of cancer in terms of gender, it is explained in table 2.

Cancer Type	**Woman (N)**	**Woman (%)**	**Male (N)**	**Male (%)**
Colon cancer	6	22.2	14	34.1
Gastric cancer	8	29.6	10	24.4
Rectal cancer	4	14.8	7	17.1
Liver cancer	3	11.1	4	9.8
Gallbladder and bile duct cancer	3	11.1	2	4.9
Pancreatic cancer	2	7.4	3	7.3
Anal cancer	1	3.7	1	2.4

Table 2: Distribution of cancer types as number and percentage of patients by gender (p = 0.921).

Considering the types of GIS cancer in terms of gender: in women, 6 (22.2%) colon cancer patients, 8 (29.6%) gastric cancer, 4 (14.8%) rectal cancer, 3 (11.1%) liver cancer, bile duct cancer bladder, and biliary tract cancers were detected in 3 (11.1%), pancreatic cancer in 2 (7.4%) and anal cancer in 1 (3.7%). In males, 14 (34.1%) colon cancer patients, 10 (24.4%) stomach cancer, 7 (17.1%) rectal cancer, 4 (9.8%) liver cancer, gallbladder and biliary tract cancer 2 It was detected in 4 (4.9%), pancreatic cancer in 3 (7.3%) and anal cancer in 1 (2.4%). When the difference between men and women in terms of GIS cancers was investigated with the chi-square test, no statistically significant difference was found ($p = 0.921$).

Serum HMGB1 levels

When the serum HMGB1 levels were examined whether they were normally distributed or not, it was determined that they were not normally distributed ($p < 0.001$). It is shown in figure 1.

Figure 1: HMGB1 scatter plot.

When female and male patients were compared in terms of serum HMGB1 levels, no statistically significant difference was found ($p = 0.299$). The mean serum HMGB1 was compared between the patient and control groups (Figure 2), and a significant difference was found since the p-value was less than 0.05. The mean serum HMGB1 levels in the patient group were 38.76 ± 29.6 (Range 11.12-160.28), and the mean serum HMGB1 levels in the control group were 11.6 ± 6.05 (range 4.62-27.97). It is shown in table 3.

Figure 2: Comparison of serum HMGB1 levels of patient and control groups ($p < 0.001$).

	Patient (n = 68) Average	Control (n = 40) Average
HMGB1 (ng/ml)	38.76 ± 29.6 (Range 11.12-160.28)	11.6 ± 6.05 (Range 4.62-27.97)

Table 3: The mean value of serum HMGB1 levels of the patient and control groups.

Mean serum HMGB1 levels were compared between disease groups (Table 1 and Figure 1). Serum HMGB1 levels averaged 11.6 ng/ml in the normal group and 36.8 ng/ml in the patient group. In cancer types, mean HMGB1 levels are 38.6 ng/ml in colon cancer, 37.2 ng/ml in stomach cancer, 49.4 ng/ml in rectum cancer, 33.03 ng/ml in pancreatic cancer, 24.2 ng/ml in liver cancer, 39.9 ng/ml in bile cancer and 58.3 ng/ml in anal cancer patients. Gastrointestinal cancer groups were compared among themselves in terms of HMGB1 levels and no significant difference was found as shown in Figure 3 ($p = 0.193$).

In the ROC analysis, it was determined that a sensitivity of 98.5%, a specificity of 90%, and a value of 19.31 could distinguish the patient and control groups as shown in figure 4 ($p < 0.001$).

Survival effect of serum HMGB1 level

While the number of gastrointestinal cancer patients was 68, 45 of these patients died during follow-up. Censored data constituted

Figure 3: Serum HMGB1 levels of GIS cancers.

Figure 4: ROC Curve of HMGB1.

34% of the patients. The mean life expectancy of the patients was 33.1 ± 18.1 months as shown in figure 5.

Figure 5: Mean survival times for patients.

When the HMGB1 level of 38.7 and above was considered as high HMGB1 levels and the patients were grouped as high and low HMGB1 levels, no difference in survival was detected as shown in figure 6 ($p = 0.219$).

Figure 6: The survival difference of patients' HMGB1 levels.

Number of individuals studied, the number of individuals who died and the percentage of censored individuals of gastrointestinal cancer types are given. Censorship represents a particular missing data in survival analysis. The type of GI cancer with the lowest survival rate is liver cancer. It is the longest living type of anal cancer. There was a difference in survival between gastrointestinal malignancies ($p < 0.001$) (Table 4, Figure 7).

	Number of Individuals Worked	Number of Deceased Individuals	Percentage of Censored Individuals
Stomach CA	18	13	27%
Rectum CA	11th	6	45%
Liver CA	7	7	0%
Colon CA	20	12	40%
Pancreatic CA	5	4	20%
Anal CA	2	0	100%
Bile CA	5	4	20%

Table 4: Complementary features of patients' survival.

Figure 7: Survival difference between gastrointestinal cancers.

Discussion

In this study, we evaluated the validity of HMGB1 as a serological biomarker for gastrointestinal cancers. Serum HMGB1 levels were significantly increased in patients with gastrointestinal cancer compared to patients without cancer.

The role of HMGB1 in cancer is complex and its function in different cancers is unclear [19]. However, HMGB1 is the focus of recent cancer research. HMGB1 plays a critical role in cancer development, progression, and metastasis by activating cancer cells, enhancing tumor angiogenesis, and suppressing host anti-cancer immunity. HMGB1 is an important target for cancer therapy [20]. Therefore, individual studies of HMGB1 levels in each cancer type are needed for a more accurate understanding of HMGB1-related mechanisms in cancer development and progression.

A recent study found that HMGB1 expression is higher in gastric tumor tissues than in normal tissues. It found that they may be useful prognostic indicators for patients with gastric cancer [21]. HMGB1 was found to be overexpressed in all colon cancer tissues [22], and HMGB1 is the diagnostic factor for this cancer. E.g; colorectal It was found that serum HMGB1 level increased 1.5 times in patients with carcinoma compared to healthy controls. As a result, colorectal show that serum HMGB1 levels are increased in a subset of carcinomas, suggesting that colorectal suggests their potential use as a supportive diagnostic marker for carcinomas [23]. In addition, HMGB1 protein deficiency has a positive effect on liver cancer cells. The reduction of this protein reduces the development of liver cancer cells. In a study, HMGB1 was found to be highly expressed in liver cancer compared to normal tissues and was also positively associated with pathological grade and distant metastases of liver cancer [24]. In this study, we measured serum HMGB1 levels of gastrointestinal cancer and healthy individuals. HMGB1 serum levels increased separately according to gastrointestinal cancer types compared to healthy individuals. We also compared the sensitivity and specificity of gastrointestinal cancer serum HMGB1 with serum HMGB1 levels in the control group. In one study, serum HMGB1 levels were found to be significantly different between disease groups and HMGB1 levels tended to increase with the progression of gastric carcinogenesis. The sensitivity and specificity of serum HMGB1 are 71% and 67%, respectively [25]. This is similar to the results of our study. In the study, we found that sensitivity of 98.5%, a specificity of 90%, and a value of 19.31 could distinguish the patient and control groups.

In our study, no distinctive difference was observed when serum HMGB1 levels were compared between GIS cancer types.

We analyzed the overall survival of cancer patients. We investigated the difference between serum HMGB1 levels and survival. In the study, it was observed that the expression of HMGB1 protein was decreased in the pancreatic tumor when compared to normal tissue and pancreatic cancer tissue. The mean survival time of the low HMGB1 group versus the high group was found to be 43 months versus 10 months [13]. However, in our study, when HMGB1 levels 38.7 and above were accepted as high HMGB1 levels, and when patients were grouped as high and low HMGB1 levels, no difference in survival was detected. Further studies are needed to evaluate the precise relationship between HMGB1 levels and gastrointestinal malignancies.

HMGB1 has been implicated as a putative danger signal involved in the pathogenesis of various non-infectious inflammatory conditions such as autoimmunity, cancer, and trauma. HMGB1 is an important prognostic factor for gastrointestinal cancers due to its role in angiogenesis, metastasis, and inflammation. Evidence for the role of HMGB1 in cancer progression, angiogenesis, invasion, and metastasis is increasing [5,26,27]. Therefore, more studies are needed to better understand the effect of HMGB1 on gastrointestinal cancer and to examine its benefits.

Conclusion

HMGB1 may be a new serological biomarker for its contribution to early diagnosis and treatment in cancers with poor prognosis and high mortality, such as the digestive system.

Acknowledgements and Ethical Approval

The approval was obtained from the Gaziantep University Faculty of Medicine Ethics Committee with the decision dated 15.01.2020 and numbered 2019/493 for this research, and it was studied in accordance with the Helsinki Declaration Rules. All patients participating in the study were informed about the study and their written consent was obtained. Thanks to the Gaziantep University Scientific Research Projects Commission with the project numbered TF.YLT.20.16. to support this study.

Declaration of Interests

None declared.

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