CLINICAL SIGNIFICANCE OF SERUM INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) AND INSULIN-LIKE GROWTH BINDING PROTEIN-3 (IGFBP-3) IN PATIENTS WITH GASTRIC CANCER

SÜLEYMAN BADEMLER[1], MURAT SARI[2], MUHAMMET ZUBEYR ÜÇÜNCÜ[3], MURAT SERILMEZ[4], SENEM KARABULUT[2]

[1]Department of Surgery, Institute of Oncology, Istanbul University, Istanbul, Turkey - [2]Department of Medical Oncology, Institute of Oncology, Istanbul University, Istanbul, Turkey - [3]Department of General Surgery, Institute of Health Sciences, Istanbul Gelişim University, Istanbul, Turkey - [4]Department of Basic Oncology, Institute of Oncology, Istanbul University, Istanbul, Turkey

ABSTRACT

Introduction: *Early diagnosis reduces mortality in gastric adenocarcinoma (GC). However, there are no markers that can be used to allow early diagnosis. The aim of the present study was to investigate clinical utility of insulin-like growth factor-1 (IGF-1) and insulin-like growth-binding protein-3 (IGFBP-3) in the diagnosis of GC.*

Materials and methods*: Hundred and fifteen patients with histopathologically confirmed diagnosis of GC and 53 age- and sex-matched healthy controls were included in our study at Istanbul University Institute of Oncology. Serum IGF-1 and IGFBP-3 levels were determined using enzyme-linked immunosorbent assay (ELISA).*

Results*: The mean age of the patients was 61 (range: 32-89) years. At the end of the median 11-month follow-up period, 75% (n=86) of the patients died. Serum IGF-1 and IGFBP-3 levels were significantly lower in the patient group than those in the control group (p=0.001). The sensitivity and specificity for IGF-1 were found to be 62.61% and 68.52%, respectively. The sensitivity and specificity for IGFBP-3 were found to be 73.91% and 62.96%, respectively. Serum IGFBP-3 levels were significantly higher in younger patients compared to those in older patients (p=0.009). The median survival was 14±3.3 months (95% CI=7.6-20.4). 3-year survival rate was 25.6% (95% CI=15.4-35.8). Large T status, high N status and metastasis were found to have a prognostic role on survival (p=0.05, p=0.05, and p=0.003, respectively). Serum IGF-1 and IGFBP-3 concentrations had no prognostic role on survival (p=0.72, p=0.41, respectively).*

Conclusion*: In our study, we showed that serum IGF-1 and IGFBP-3 levels could be used for early diagnosis of GC. We found that these two biomarkers have good sensitivity and specificity in clinical practice.*

Keywords*: Gastric cancer, serum, IGF-1, IGFBP-3.*

DOI: 10.19193/0393-6384_2018_6_320

Received *July 17, 2018;* ***Accepted*** *Septemper 20, 2018*

Introduction

Gastric adenocarcinoma (GC) is the second most common cause of cancer-related deaths. The incidence of gastric adenocarcinoma is heterogeneous based on the geographical distribution, and occurs frequently, particularly in younger populations in Europe, South Asia and USA. This type of tumor, which is frequently seen in younger patients, is often recognized in the advanced stage as it shows its symptoms in the late periods. Invasion into the surrounding tissue indicates poor prognosis. In patients with distant organ metastases, 3-year survival rate has been reported to be 8%. However, 5-year survival rate is as high as 80% in patients diagnosed in the early stages[1]. In this type of cancer, early diagnosis, early treatment and close follow-up may reduce mortality. However, there is a need for reliable markers that can be used as a rapid test in the early diagnosis[2,3]. Although not specific to GC, biomarkers such as serum carbohydrate antigen (CA) 19-9 and CA 72-4 levels are utilized in

the diagnosis of GC along with imaging studies and pathological diagnosis. CA 19-9 is rather used for the purpose of monitoring prognosis. The sensitivity and specificity of these two markers are different from each other. Combined use of these two markers is recommended for higher specificity and sensitivity[3,4]. There is no more sensitive marker that can be used alone in the diagnosis and follow-up of GC and there are ongoing studies on this subject.

Insulin-like growth factor (IGF) family has mitogenic and anabolic effect on normal tissue. In addition, it is a protein complex that plays an active role in the proliferation of cancer cells and it prevents cell apoptosis. IGF family consists of IGF 1-6, IGFR 1-2 (Insulin-like growth factor receptor 1-2) and IGFBP 1-3 (Insulin-like growth factor binding protein 1-3). Preclinical studies have reported that increased IGF expression in the tumor tissue can be used as a significant biomarker[5-8]. Among its known sub-types, increased IGF-1 expression was reported to be the most significant finding for cancers and that it can provide information about metastasis, prognosis and chemotherapy resistance of tumors originating from the mesenchymal cells[6].

IGFBP complex is a marker that assists transport of IGF-1 in the blood and triggers cell apoptosis. IGFBP complex is of three different types. IGFBP-1 and 2 are involved in the regulation of IGF-1, whereas IGFBP-3 has a role in reducing the binding functions in circulating IGF. 90% of circulating IGF-I is bound to IGFBP-3. In the studies on IGF family in non-GC tumors, high IGF-1 and low IGFBP-3 levels have been shown to be associated with an increased risk of prostate, colorectal and breast cancer (5,7,9,10). However, the studies on the use of this family as a clinical biomarker in GC are limited and include data involving Japanese patients (11). For this reason, clinical outcomes in different ethnic groups are unknown. In our study, we aimed to investigate the clinical significance of serum IGF-1 and IGFBP-3 levels in patients with GC.

Materials and Methods

Patients

Hundred and fifteen patients with pathologically established diagnosis of GC that were followed at Istanbul University Institute of Oncology between 2013 and 2015 and 53 healthy controls were included in the study. Patients who had previously received chemotherapy at another center, those who did not want to participate in the study, and those who were lost to follow-up and continued their treatment at another center were excluded from the study. Age, gender and clinicopathologic parameters of the patients were recorded from the patient files. Disease staging was performed based on the International Union Against Cancer TNM classification. Computed tomography (CT), magnetic resonance imaging (MRI) and/or positron emission tomography (PET/CT) were used as imaging methods.

Blood samples and study method

Blood samples were collected from the patients and control subjects who provided consent for the study upon admission to our clinic. The sera separated from the blood samples after centrifugation were stored at -80˚C until analysis. Serum IGF-1 and IGFBP-3 levels were measured using Immulite 2000 system (all from Siemens Healthcare Diagnostics Products Ltd., Sudbury, UK). This system is based on solid phase enzyme-linked chemiluminescence (EIA) method. After the samples were diluted, serum IGF-1 and IGFBP-3 levels were automatically studied.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA). The fitness of the variables to normal distribution was evaluated by analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics included median and range for variables without normal distribution. The variables were compared between the groups using the Mann-Whitney U test. Overall survival (OS) was defined as the time from the start of treatment to last control visit or death. Survival analysis was performed using Kaplan-Meier method. Differences in survival were analyzed using the log-rank test. A P value of ≤ 0.05 was considered statistically significant.

Results

The mean age of the patients was 61 (range: 32-89) years, and 69% were male. When the patients were grouped according to the disease stage, 18 patients had stage 1-2, 60 patients had stage 3, and 37 patients had stage 4 disease. Based on this data, 32% of the patients had metastatic disease at the time of diagnosis. Patient characteristics are reported in Table 1.

Variables	n
No. of patients	115
Age (years) Median (range)	61 (32-89)
Gender Male/female	79/86
Site of lesion Antrum/non-antrum	30/85
Angio-lymphatic invasion* Yes/no	44/9
Perineural invasion* Yes/no	45/8
Pathologic tumor (pT) stage* 1/2/3/4	3/7/24/51
Pathologic node (pN) stage* 0/1/2/3	12/17/9/22
Metastasis Yes/no	37/85

Table 1: Characteristics of the patients and disease.
**Patients with unknown data concerning the variables are not included in the analysis*

There was a statistically significant difference between the study patients and the controls in terms of IGF-1 and IGFBP-3 levels (Table 2). Both levels were significantly higher in the control group. Serum IGF-1 levels in patients with GC were significantly lower than those in the control group. In patients with GC, serum IGF-1 level was 116.63 ng/ml, whereas it was 158.63 ng/ml (p=0.001) in the control group. Serum IGFBP-3 levels in patients with GC were significantly lower than those in the control group. Serum IGFBP-3 level was 3.24 ng/ml in patients with GC, whereas it was 4.44 ng/ml (p=0.001) in the control group.

Markers	Patients (n=115)	Healthy controls (n=53)	p
IGF-1 (median±SD) (ng/ml)	116.63±73.32	158.63±62.22	0.001**
IGFBP-3 (median±SD) (ng/ml)	3.24±1.40	4.44±1.36	0.001**

Table 2: The values of serum IGF-1 ve IGFBP-3 levels in GC patients and healthy controls.
***p≤ 0.05,*
(Abbreviations: GC; Gastric Cancer, IGF-1; Insulin-Like Growth Factor-1, IGFBP-3; Insulin-Like Growth Factor Binding Protein-3)

Receiver Operating Characteristics (ROC) analysis for IGF-1 revealed that the Area Under the Curve (AUC) value was 70.6% and the cut-off point was 124.5. Based on this cut-off point, sensitivity was 62.61%, specificity was 68.52%, positive predictive value (PPV) was 80.90% and negative predictive value (NPV) was 46.25%. The rate of correct classification was found to be 64.50%. ROC analysis for IGFBP-3 revealed that AUC value was 74.3% and the cut-off point was 4.03. Based on this cut-off point, sensitivity was 73.91%, specificity was 62.96%, PPV was 80.95% and NPV was 53.12%. The rate of correct classification was found to be 70.41% (Table 3, Figure 1).

	IGF-1	IGFBP-3
AUC (%95 CI)	0.706 (0.624-0.788)	0.743 (0.664-0.822)
Cut-off point	124.5	4.03
Sensitivity (%95 CI)	62.61 (53.10-71.45)	73.91 (61.90-81.66)
Specificity (%95 CI)	68.52 (54.45-80.48)	62.96 (48.74-75.71)
PPV (%95 CI)	80.90 (73.60-86.55)	80.95 (74.70-85.95)
NPV (%95 CI)	46.25 (38.98-53.68)	53.12 (43.92-62.12)
Correct classification rate (%95 CI)	64.50 (56.78-71.69)	70.41 (62.92-77.18)

Table 3: ROC analysis results.
(Abbreviations: IGF-1; Insulin-Like Growth Factor-1, IGFBP-3; Insulin-Like Growth Factor Binding Protein-3, AUC; Area under the curve; PPV; positive predictive value, NPV; negative predictive value, CI; confidence interval)

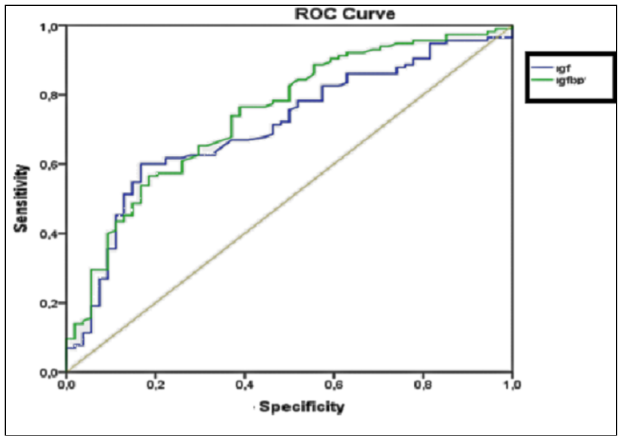

Fig. 1: ROC curves.

Serum IGBP-3 levels in younger patients and elderly patients were 3.57 and 2.86 ng/ml, respectively (p=0.009). Apart from significantly higher IGFBP-3 levels in younger patients, there was no significant difference between clinicopathologic parameters and serum IGF-1 and IGFBP-3 levels (p>0.05) (Table 4).

The mean duration of follow-up was 11 (range: 1-49) months. At the end of follow-up period, 75% (n=86) of the patients died due to the disease. The median survival was 14±3.3 months

(95% CI=7.6-20.4). 1-year survival rate was 55.8% (95% CI= 45.0-66.6). 3-year survival rate was %25.6±5.2 (95% CI=15.4-35.8). Large T status, high N status and metastasis were found to have a prognostic role on survival (respectively; p=0.05, p=0.05, and p=0.003). Serum IGF-1 and IGFBP-3 concentrations had no prognostic role on survival (respectively; p=0.72, p=0.41) (Figure 2, 3), (Table 5).

Variables	n	IGF-1 (ng/mL) Median (±SD)	p	IGFBP-3 (ng/ml) Median (±SD)	p
Age patients		0.13			
Young (<60)	54	106.50±69.18	0.13	3.57±1.25	0.009**
Older (>60)	61	92.80±76.60		2.86±1.49	
Sex		0.59			
Male	79	102.00±78.36	0.59	3.08±1.50	0.34
Female	36	94.50±61.30		3.28±1.20	
Localization					
Antrum	30	92.90±71.83	0.54	3.26±1.48	0.54
Non-antrum	85	97.80±79.16		2.87±1.38	
Angio-lymphatic invasion					
Yes	44	104.50±67.45	0.32	3.12±1.19	0.50
No	9	107.00±78.19		3.30±0.91	
Perineural invasion					
Yes	45	103.00±64.65	0.16	3.08±1.71	0.20
No	8	172.50±84.50		3.88±0.95	
pT stage					
Small (1-2)	10	111.50±34.65	0.57	3.34±0.78	0.35
Large (3-4)	75	101.00±73.62		3.07±1.38	
pN stage					
Low (1-2)	29	107.00±68.08	0.48	2.94±1.06	0.30
High (3)	31	170.50±84.87		3.54±1.54	
Metastasis					
Yes	37	94.20±77.48	0.43	3.15±1.51	0.74
No	85	102.50±71.63		3.16±1.37	

Table 4: Results of comparisons between the IGF-1 and IGFBP-3 levels assays and various clinicopatological parameters.
***p≤ 0.05*
(Abbreviations: IGF-1; Insulin-Like Growth Factor-1, IGFBP-3; Insulin-Like Growth Factor Binding Protein-3)

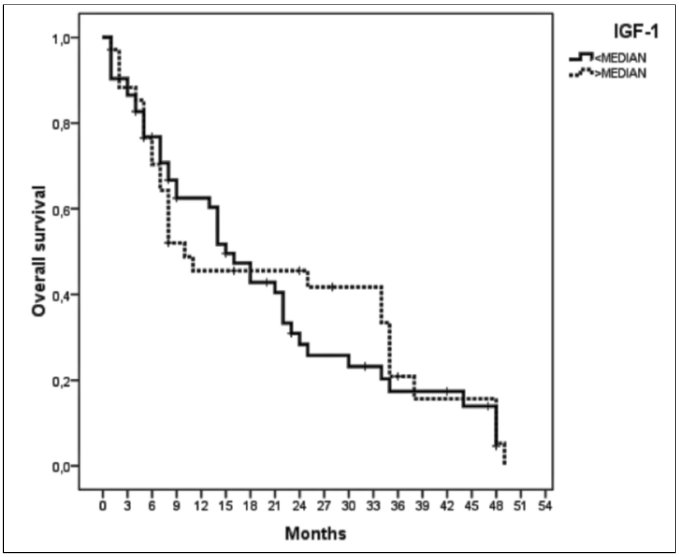

Fig. 2: Overall survival curves in GC patients according to serum IGF-1 levels (p=0.72)
Abbreviations: IGF-1; Insulin-Like Growth Factor-1

Discussion

Early diagnosis in GC increases survival and decreases mortality. The attempts have been made to discover new tumor markers with high specificity that can be used in the early diagnosis in lieu of current tumor markers[3,4].

The use of IGF and IGF-dependent markers as a tumor marker in GC has been a popular research topic in recent years. It is emphasized that these markers, which are found to play a role in the cell survival, proliferation, differentiation, apoptosis, metastasis, angiogenesis and pathogenesis, can also provide significant information about cancer cells. IGF and other markers include IGF receptors and IGF binding proteins (IGFBP) in the blood. IGF and receptor IGFBPs are secreted from different subtypes of IGFs and other markers in the body[5,6,9,12]. Another study by Kuang et al. reported that IGF levels in tumor tissues might be different even from each other[13]. For example, in mesenchymal tumors, IGF-II is secreted in higher amounts, whereas in GC cancers, IGF-1 and its receptors and IGFBP are more pronounced[2].

Our study will contribute to the studies on the identification of reliable tumor markers that can be used in the early diagnosis of GC. In this regard, we tried to find out whether IGF-1 and IGFBP, which are more specifically addressed for GC tumors in the early diagnosis of GC patients, could be used as reliable tumor markers by analyzing the data of patient and control groups. In our study, statistically significant difference was found between low IGF-1 and IGFBP-3 levels measured in sera of patients with GC and the levels measured in sera of the control group (p=0.001). Although these two biomarkers are secreted into the blood by many tissues, mainly by the liver, their levels in serum and tissue are increased especially in some tumoral formations[2,14]. However, the fact that, contrary to other tumors, IGF and other markers are released from many tissues, but not from GC tissue, suggests that GC can be used for early diagnosis and to predict survival[2,7,9,12,15-17]. These data in the literature are consistent with the findings of our study and supports that low IGF-1 and IGFBP-3 levels can be used for early diagnosis in GC.

In our study, serum IGF-1 and IGFBP-3 levels of patients with GC did not significantly correlate

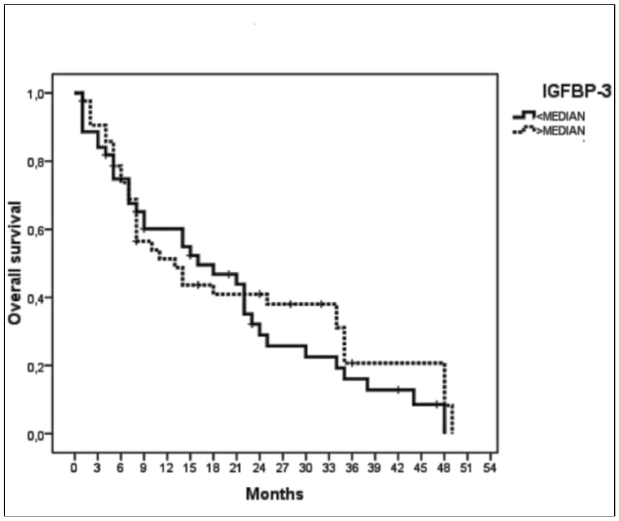

Fig. 3: Overall survival curves in GC patients according to serum IGFBP-3 levels (p=0.41).
Abbreviations: IGFBP-3; Insulin-Like Growth Factor Binding Protein-3

Parameters	Survival Median (±SD) (months)	3-year Survival (%) (±SD)	p
All patients	14.0 (3.3)	55.8 (5.5)	
Age patients			
Young (<60)	21.0 (5.6)	20.2 (7.1)	0.76
Older (>60)	13.0 (2.7)	17.6 (6.6)	
Sex			
Male	15.0 (5.0)	19.7 (6.1)	0.61
Female	14.0 (4.9)	16.7 (7.6)	
Localization			
Antrum	15.0 (6.4)	21.9 (10.3)	0.62
Non-antrum	14.0 (5.0)	24.0 (6.9)	
Angio-lymphatic invasion			
Yes	24.0 (6.5)	25.4 (8.6)	0.18
No	35.0 (4.0)	60.0 (21.9)	
Perineural invasion			
Yes	24.0 (6.5)	28.2 (8.7)	0.20
No	35.0 (4.0)	50.0 (25.0)	
pT stage			
Small (1-2)	38.0 (8.0)	66.7 (19.2)	0.05**
Large (3-4)	21.0 (3.2)	16.3 (5.8)	
pN stage			
Low (1-2)	30. 0 (8.2)	29.6 (11.1)	0.05**
High (3)	14.0 (6.0)	0.0	
Metastasis			
Yes	7.0 (1.4)	14.7 (7.2)	0.003*
No	22.0 (4.6)	22.2 (6.3)	
IGF-1			
<Median	15.0 (2.0)	17.4 (6.0)	0.72
>Median	10.0 (7.0)	20.9 (7.9)	
IGFBP-3			
<Median	16.0 (5.8)	16.1 6.4	0.41
>Median	13.0 (3.7)	20.7 (7.1)	

Table 5: Univariate analyses of overall survival.
Abbreviations: SD; Standard Deviation, IGF-1; Insulin-Like Growth Factor-1, IGFBP-3; Insulin-Like Growth Factor Binding Protein-3

with T stage, N stage and pathological stage of the tumor. This data is consistent with the study by Matsubara et al. who indicates that there is no significant difference between IGFR and prognosis[14].

Studies have reported that, as in many cancer types, increased IGF, IGFBP-3 and IGF receptor levels in the tumor tissue in GC significantly correlate with the presence of metastasis and poor prognosis[2,3,7,17]. In our study, no correlation was observed between the stage and serum biomarker levels of patients with GC patients, and this result is inconsistent with the data in the literature. This may be explained by the limited number of early stage (stage 1-2) patients and the lack of complete homogeneity between the stages. However, since this is the patient distribution for the time interval we determined during the study, we present this result in this manner.

In our study, no statistically significant difference was found between the below- and above-median serum IGF-1 and IGFBP-3 levels and the survival (p=0.88, p=0.25, respectively). In the only study in the literature that showed prognostic significance of IGFBP-3, Xue et al. showed that higher IGFBP-3 levels could indicate favorable five-year survival[17]. Further research into this topic should clarify whether these two biomarkers can be a prognostic marker in patients with GC.

It has been reported that since IGF-1, 2 and IGFBP-3 are not different from each other in the diagnosis of cancer patients and that they can be used alone as a bio-marker; however, it is emphasized that combined evaluation of these markers would increase sensitivity and specificity[2,12,18]. In addition, tumor markers to be used in cancer diagnosis should have high sensitivity and specificity. In the reported studies, the data on the reliability of these markers are based on ROC analysis. Based on these two principles, serum levels of these two markers in the present study were evaluated jointly and the reliability of each marker was calculated separately by ROC analysis. ROC analysis revealed that the sensitivity and specificity for IGF-1 test were 62.6% and 68.5%, respectively. For IGFBP-3, they were found to be 73.9% and 63.0%. When we compared these data with the data for serum markers used in clinical practice, in the study by Ningss et al. the sensitivity rate for CEA ve CA19-9 - tumor markers which are frequently used in the diagnosis and follow-up of GC patients - was higher than that of our study, whereas the specificity rate was lower[4].

We aimed to evaluate the IGF-1 and IGFBP-3 levels in serum instead of tissue in order to make our study easier and faster. In this study, we showed that measurement of serum IGF-1 and IGFBP-3

levels in the diagnosis of GC is an easy, fast, effective and reliable method. There is a need for prospective studies with larger and homogeneous patient groups on clinical utility of these two markers in patients with GC.

References

1) Ikoma N, Blum M, Estrella JS, Das P, Hofstetter WL, et al. Badgwell-Evaluation of the American Joint Committee on Cancer 8th edition staging system for gastric cancer patients after preoperative therapy. Gastric Cancer 2018; 21: 74-83.
2) Rahman OA. Insulin-like growth factor pathway aberrations and gastriccancer; evaluation of prognostic significance and assessment of therapeutic potentials. Med Oncol 2015; 32: 431.
3) Gryko M, Kis´luk J, Cepowicz D, Zin´czuk J, Kamocki Z, et al. Expression of insulin-like growth factor receptor type 1 correlate with lymphatic metastases in human gastric cancer. Pol J Pathol 2014; 65(2): 135-40.
4) Ning S, Wei W, Li J, Hou B, Zhong J, et al. Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 levels in gastric and colorectal cancer patients. J Cancer. 2018; 9(3): 494-501.
5) Furstenberger G, Senn HJ. Insulin-like growth factors and cancer. Lancet Oncol 2002; 3(5): 298-302.
6) Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004; 4(7): 505-18.
7) Li H, Batth IS, Qu X, Xu L, Song N, et al. IGF-IR signaling in epithelial to mesenchymal transition and targeting IGFIR therapy: overview and new insights. Mol Cancer 2017; 16: 6.
8) Miller BS, Yee D. Type I insulin-like growth factor receptor as a therapeutic target in cancer. Cancer Res 2005; 65(22): 10123-7.
9) Baxter RC. Signalling pathways involved in antiproliferative effects of IGFBP-3: a review. Mol Pathol 2001; 54(3): 145-48.
10) Douglas JB, Silverman DT, Pollak MN, Tao Y, Soliman AS, et al. Serum IGF-I, IGF-II, IGFBP-3, and IGF-I/IGFBP-3 Molar Ratio and Risk of Pancreatic Cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Cancer Epidemiol Biomarkers Prev 2010; 19(9): 2298-306.
11) Ennishi D, Shitara K, Ito H, Hosono S, Watanabe M, et al. Association between insulin-like growth factor-1 polymorphisms and stomach cancer risk in a Japanese population. Cancer Sci 2011; 102(12): 2231-5.
12) Lee DY, Yang DH, Kang CW, Kim SJ, Joo CU, et al. Serum insulin-like growth factors (IGFs) and IGF binding protein (IGFBP)-3 in patients with gastric cancer IGFBP-3 protease activity induced by surgery. J Korean Med Sci 1997; 12(1): 32-9.
13) Kuang RG, Wu HX, Hao GX, Wang JW, Zhou CJ. Expression and significance of IGF-2, PCNA, MMP-7, and a-actin in gastric carcinoma with Lauren classification. Turk J Gastroenterol 2013; 24(2): 99-108.
14) Matsubara J, Yamada Y, Nakajima TE, Kato K, Hamaguchi T, et al. Clinical Significance of Insulin-Like Growth Factor Type 1 Receptor and Epidermal Growth Factor Receptor in Patients with Advanced Gastric Cancer. Oncology 2008; 74: 76-83.
15) Wang HB, Zhou CJ, Song SZ, Chen P, Xu WH, et al. Evaluation of Nrf2 and IGF-1 expression in benign, premalignant and malignant gastric lesions. Pathol Res Pract 2011; 207(3): 169-73.
16) Khandwala HM, Mccutcheon IE, Flyvbjerg A, Friend K. The Effects of Insulin-Like Growth Factors on Tumorigenesis and Neoplastic Growth. Endocr Rev 2000; 21(3): 215-44.
17) Xue M, Fang Y, Sun G, Zhuo W, Zhong J, et al. IGFBP3, a Transcriptional Target of Homeobox D10, Is, Correlated with the Prognosis of Gastric Cancer. PLOS one 2013; 8: 12.
18) Weroha S. John, Haluska Paul. IGF System in Cancer. Endocrinol Metab Clin North Am 2012; 41(2): 335-50.

Corresponding author
SENEM KARABULUT
E-mail: drsenemkarabulut@gmail.com
(Turkey)