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Isfahan University of Medical Sciences

Original Article

Clinicopathological correlation of insulin-like growth factor binding protein 3 and their death receptor in patients with gastric cancer

Amir Ansari[1], Ali Gheysarzadeh[2], Ali Sharifi[3], and Mohammad Reza Mofid[1,*]

[1]*Department of Clinical Biochemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.*
[2]*Department of Biochemistry, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.*
[3] *Department of Internal Medicine, School of Medicine, Shahid Mostafa Khomeini Hospital, Ilam University of Medical Sciences, Ilam, Iran.*

Abstract

Background and purpose: The insulin-like growth factor binding protein 3 (IGFBP-3) and its novel death receptor (IGFBP-3R) have been exhibited to have tumor suppressor effects. Despite their prognostic value in some cancers, they have not been elucidated in gastric cancer.
Experimental approach: We collected 68 samples from patients with gastric cancer. IGFBP-3 and IGFBP-3R expression levels were evaluated with quantitative real-time polymerase chain reaction (RT-PCR) and western blotting in patients. The relationship between prognostic factors and IGFBP-3/IGFBP-3R expression was also evaluated.
Findings/Results: Our results showed that IGFBP-3 and IGFBP-3R expression was reduced significantly in tumor tissues. We found that there was an association between the reduction of IGFBP-3 with lymph node metastasis and tumor-node-metastasis (TNM) staging. Besides, IGFBP-3R expression was associated with tumor size, lymph node metastasis, differentiation, and TNM classification. Interestingly, we presented that the downregulation of IGFBP-3R was stage-dependent. In survival analysis, our findings showed that low levels of IGFBP-3R mRNA expression exhibited a close correlation with survival rate.
Conclusion and implications: The findings of this study showed that the expression levels of IGFBP-3 and IGFBP-3R are valuable prognostic factors. Despite the potential of IGFBP-3, IGFBP-3R plays a significant role as a prognostic factor in gastric cancer. However, these findings need to be developed and confirmed by further studies.

Keywords: Gastric cancer; IGFBP-3; IGFBP-3R; Prognostic factor; TNM classification.

INTRODUCTION

Gastric cancer (GC) is known as the fourth most common malignancy and the third cause of cancer-related death all over the world (1). The 5-year survival of gastric cancer patients with adjuvant treatment in some cases can be extended to than 31% (2). Poor prognosis, inconspicuous symptoms, and lack of effective diagnosis in an early stage of GC. GC identification usually takes place just after its symptoms are represented in a patient with advanced stage (3). Therefore, understanding the molecular mechanism of cancer progression can improve the development of novel treatment strategies (4).

Insulin-like growth factor-binding protein 3 (IGFBP-3) has been identified as a conserved and multi-functional protein that can bind to 80% of IGF-I and IGF-II with high affinity and regulate IGF signaling (5). The main function of IGFBP-3 has been identified in a wide variety of cancers (6). This is the most abundant secreted protein of the IGFBPs family and participates in an IGF-dependent/independent manner.

*Corresponding author: M.R. Mofid
Tel: +98-3137927047, Fax: +98-3136680011
Email: mofid@pharm.mui.ac.ir

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	Website: http://rps.mui.ac.ir
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IGFBP-3 has been shown to induce apoptosis in an IGF-independent action (7). To determine the specific receptor of IGFBP-3, for the first time, Kim *et al.* demonstrated that IGFBP-3 can bind to the cell surface-specific receptor by yeast two-hybrid screening (8). Also, it was demonstrated that the IGFBP-3 receptor (IGFBP-3R) is widely expressed in most tissues. It contains 240 amino acid residues in length and interacts with IGFBP-3 in the extracellular membrane (9). IGFBP-3R, a single-span membrane protein, was identified as a novel cell death receptor by Ingerman and colleagues (9). They showed that the central domain of IGFBP-3 is critical for binding to IGFBP-3R and activating caspase-8 can induce apoptosis in unconventional ways. Recently, it has been shown that impairing of the IGFBP-3/IGFBP-3R axis occurs in many malignancies (10). IGFBP-3 expression appears to be suppressed in many cancers, due to some epigenetic alteration like hypermethylation (11). Besides, IGFBP-3R expression is significantly reduced in invasive breast ductal carcinoma, pancreatic ductal adenocarcinoma, and prostate tumor cells (9,12). Thus, investigation of the IGFBP-3/IGFBP-3R axis may provide prognostic and therapeutic value for primary diagnosis/staging and gastric cancer treatment.

In the present study, the expression of IGFBP-3 and IGFBP-3R was evaluated using quantitative-polymerase chain reaction (qRT-PCR) and western blotting in GC tumor tissues in comparison with their normal adjacent tissues to serve as the potential prognostic marker of GC. The association of mentioned protein expression with clinicopathological features and overall survival was evaluated as well.

MATERIALS AND METHODS

Tissue samples

This study included 68 pairs of GC samples collected from surgical resection between April 2014 and September 2016 obtained from Iran National Tumor Bank (INTB, Tehran, Iran). All samples were snap-frozen in liquid nitrogen and were evaluated by two independent pathologists blinded. Patients were written an informed consent form, procedures were according to the ethical standards of the institutional and/or national research committee of the 2013 Helsinki Declaration and the investigation was approved by Ethical Committee Members of the Medical University of Isfahan (Ethic code number: IR.MUI.REC.1396,3,386). All specimens without any treatments like radiotherapy or chemotherapy were enrolled in the study. Normal samples were removed from the marginal zone of cancer tissue (> 5 cm apart from a tumor) and used as a control. Samples are characterized according to the American Cancer Society and tumor-node-metastasis (TNM) classification system guidelines (13). The clinicopathological features of the samples are summarized in Table 1.

Chemicals, reagents, and antibodies

RNA extraction reagents (RNXTM-plus) and DNaseI were provided from Cinnagen (Cinnagen, Tehran, Iran). cDNA synthesis kit and the enhanced chemiluminescent detection system (ECL) were purchased from Takara (Takara Shuzo Co. Ltd., Tokyo, Japan). All primers and high ROX™ SYBR Green master mix were obtained from Ampliqon (Ampliqon, Herlev, Denmark). Primary sheep polyclonal anti-IGFBP-3R (AF7556-SP) and secondary donkey anti-sheep IgG horseradish peroxidase (HRP)-conjugated antibody (HAF016) were purchased from R&D (R&D Systems™, Minneapolis, USA). Mouse monoclonal antiβ-actin and anti-IGFBP-3, secondary mouse anti-goat IgG HRP-conjugated was obtained from Santa Cruz (Santa Cruz Biotechnology, CA, USA). Electrophoresis reagents and materials were provided by Bio-Rad (Hercules, CA, USA). Other chemicals and reagents were obtained from Sigma Aldrich (St. Louis, MO, USA).

RNA extraction and qRT-PCR

Total RNA was extracted from all tissues by using RNXTM-plus according to the manufacturer's protocol. Frozen tissues (20-30 mg) homogenized by the bead-milling method in 1 mL of RNXTM-plus reagent as described previously (14). Briefly, after homogenizing the samples, supernatant was harvested, and chloroform was added and mixed. After incubation on ice, the mixture was centrifuged at 12000 rpm at 4 °C for 15 min. For RNA isolation, supernatants were transferred to an RNase-free tube, an equal volume amount of isopropanol was added, and incubated on ice for 15 min. After centrifuging at 12000 rpm for 15 min, the supernatant was discarded, 75% ethanol was added, then the pellet was dislodged and centrifuged at 4 °C for 8 min at 7500 rpm. Then, supernatants were discarded, pellets were dried at room temperature and dissolved in 50 µL of diethyl pyrocarbonate-treated water. For the elimination of DNA, the suspension was treated with DNaseI. Quantity and purity of RNA were then determined by ultraviolet spectrophotometer (BioTek, Winooski, VT, USA) by using A260/A280 ratio and gel agarose electrophoresis, respectively.

Total RNA (2 µg) was used for cDNA synthesis according to the kit's protocol. RT-PCR was performed utilizing an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA). The sequences of primers are provided in Table 2.

Table 1. Association of clinicopathological features with IGFBP-3 and IGFB-3R mRNA relative expression in 68 patients with gastric cancer. The data were analyzed using Pearson chi-square tests.

Parameters	**Number of patients**	**IGFBP-3 mRNA expression**		***P*-value**	**IGFBP-3R mRNA expression**		***P*-value**
		Low	**High**		**Low**	**High**	
Age				0.250			0.163
< 61	28 (41.2%)	12	16		12	16	
≥ 61	40 (58.2%)	28	12		24	16	
Sex				0.536			0.254
Male	54 (79.4%)	32	22		30	24	
Female	14 (20.6%)	8	6		6	8	
Tumor size				0.146			0.004
< 6	34 (50%)	14	20		12	22	
≥ 6	34 (50%)	26	8		24	10	
Lymph node invasion				< 0.001			< 0.001
Positive	50 (73.5%)	34	16		32	18	
Negative	18 (26.5%)	6	12		4	14	
Differentiation				0.186			0.002
Poor	18 (26.5%)	8	10		0	18	
Moderate	40 (58.8%)	26	14		30	10	
High	10 (14.7%)	6	4		6	4	
Tumor-node-metastasis stage				< 0.001			0.005
IB+II	26 (38.2%)	10	16		10	16	
IIIA	18 (26.5%)	10	8		8	10	
IIIB+IV	24 (35.5%)	20	4		18	6	

IGFBP, Insulin-like growth factor binding protein; IGFBP-3R, insulin-like growth factor binding protein-3 receptor.

Table 2. Sequences of the primers.

Gene's name	**Sequence (5′-3′)**
IGFBP-3	Forward: GGTGTCTGATCCCAAGTTCC
IGFBP-3	Reverse: ACCATATTCTGTCTCCCGCT
IGFBP-3R	Forward: TGACCACCTTGAACTTCG
IGFBP-3R	Reverse: GCAGAAGATCCTTTCAATC
GAPDH	Forward: CAGCCTCAAGATCATCAGC
GAPDH	Reverse: GGCAGTGATGGCATGGACT

IGFBP, Insulin-like growth factor binding protein; IGFBP-3R, insulin-like growth factor binding protein-3 receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

The final volume of the reaction mixture (10 µL) contained 1 ng of cDNA template, 200 nM each of sense and antisense primers, and 5 µL of 2X SYBR Green PCR. The reaction conditions were as follows: after an initial hot start (95 °C) for 10 min, amplification was performed for 40 cycles containing denaturation for 10 s at 94 °C, annealing for 30 s at 50 °C, and extension for 40 s at 72 °C. The amplification kinetics were recorded as sigmoid progress curves for which fluorescence was interoperated against the number of amplification cycles. The threshold cycle number was used to define the initial amount of each template. Fluorescence readings were carried out in every amplification cycle, using StepOnePlus (Applied Biosystems, Foster City, CA, USA). All measurements were performed in triplicate. The sizes of the amplified fragments were confirmed by agarose gel 2% electrophoresis. All results normalized with GAPDH as an internal control and then fold changes were analyzed according to the $2^{-\Delta\Delta Ct}$ method:

$\Delta\Delta Ct$ = (Ct gene of interest $_{\text{cancerous tissues}}$ - Ct GAPDH $_{\text{cancerous tissues}}$) - (Ct gene of interest $_{\text{normal tissue}}$ - Ct GAPDH $_{\text{normal tissue}}$).

Western blotting

IGFBP-3 and IGFBP-3R protein expression were evaluated with western blotting as described previously (12). In summary, 100 mg of tissues were homogenized by the bead-milling method in 1 mL ice-cold radioimmunoprecipitation assay buffer (50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 15 mM $Na_4P_2O_7$, 20 mM NaF, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 6 mM egtazic acid, 100 mM glycerol 3-phosphate, 1% NP-40 and 1% sodium deoxycholic acid supplemented with 0.5% freshly protease and phosphatase inhibitors cocktail (Melford, Ipswich, UK). The lysates were harvested with centrifugation (14,000 rpm) at 4 °C for 25 min and the supernatant was stored at -80 ℃.

Before sample loading, the protein concentrations were measured by the Bradford method. All protein samples were incubated with Laemmli buffer at 100 °C for 5 min, and an equal amount (40 µg) of total proteins were separated by electrophoresis in a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Ready Gel, Bio-Rad, USA). Following, transferred to a polyvinylidene fluoride membrane (Amersham Pharmacia Biotech, United Kingdom), membranes were blocked Tris-buffered saline -0.1% Tween 20 (TBS-T) containing 5% non-fat dried milk for 2 h at room temperature. After 3 times washing with TBS-T, membranes were incubated overnight with each primary antibody at 4 °C (1:1000 in TBS-T and 0.1% bovine serum albumin (BSA)). After washing three times with TBS-T for 5 min, the membranes were incubated in secondary antibodies (1:2500 in TBS-T and 0.1% BSA). After three times washing with TBS-T protein bands were detected with the ECL reagent. All bands were normalized by β-actin as the internal control. The relative intensity of all bands was quantified by densitometry, using the Image J software (NIH, Bethesda, MA, USA).

Statistical analysis

The comparison of RNA and protein relative expression levels between the normal and the tumor tissues was assessed with a paired Student's t-test. The One-way ANOVA, followed by Tukey HSD, and independent sample T-test were used to evaluate the relationship between clinicopathological parameters and IGFBP-3/IGFBP-3R expression. The overall survival (OS) rates were calculated by the Kaplan-Meier method and differences in survival rates between subgroup patients (high and low expression) were analyzed with the log-rank test. The categorical data were analyzed using the Pearson chi-square test. All experiments were performed in triplicate and data are presented as mean ± SEM. Statistical significance was determined at the level of $P < 0.05$. All data were analyzed with SPSS 22 (SPSS, Chicago, IL, USA).

RESULTS

Patients

All clinicopathological features of patients are summarized in Table 1. This study contains 54 males and 14 females. The median age of patients with GC was 62 ± 10 years (ranging from 33 to 76 years). The tumor size was classified into two groups based on the mean (6 cm), there are three grades for tumors categorized as well, moderate and poor differentiation (grades 1, 2, and 3, respectively), the stage was classified in IB+II, IIIA, and IIIB+IV.

IGFBP-3 expression was reduced in GC tumor tissue in comparison with normal adjacent tissue

We measured the IGFBP-3 expression in 68 paired GC tissues. The results of qRT-PCR indicated that the mRNA relative expression of IGFBP-3 was markedly reduced in cancerous tissue with a fold change of 0.47 ± 0.04 compared to normal tissue (Fig. 1A). Analysis based on different TNM stages and fold changes indicated that IGFBP-3 expression decreased in IIIA and IIIB+IV stages compared to the normal group (Fig 1B). Also, relative protein expression of IGFBP-3 was evaluated with western blotting (Fig. 2A). The results indicated that the protein expression was significantly reduced compared with the paired normal tissue (fold change of 0.85 ± 0.13, Fig. 2B). In analyzing the pattern of protein expression, the reduction of IGFBP-3 protein in stage IB+II was not significant, but in IIIA and IIIB+IV stages were statistically significant compared to normal tissue (Fig. 2C).

IGFBP-3R expression reduced in GC tumor

Our findings indicated that mRNA relative expression of IGFBP-3R was markedly reduced in GC tumors in comparison with normal tissue (fold change of 0.49 ± 0.05, Fig. 3A). TNM stage analysis revealed that IGFBP-3R mRNA expression was significantly reduced stage-dependently in IB+II (0.69 ± 0.12, P=0.003), IIIA and IIIB+IV stages (Fig. 3B). In western blotting analysis (Fig. 4A), IGFBP-3R protein expression was significantly reduced in GC tumor tissue compared to normal tissue (fold change of 0.53 ± 0.02, Fig. 4B). Besides, analysis of IGFBP-3R protein expression pattern indicated that it depends on stages IB+II and IIIA. However, the decreasing expression of this protein is not significant in the IIIB+IV stage (Fig. 4C).

Association between IGFBP-3 and IGFBP-3R expression with clinicopathological parameters in GC patients

The relationship between the relative expression of IGFBP-3 and IGFBP-3R with clinicopathological variables was analyzed by one-way ANOVA and independent sample T-test, listed in Table 1. The analysis revealed that IGFBP-3 expression was strongly associated with lymph node invasion and TNM stage. Moreover, analyzing IGFBP-3R expression revealed that the low-level expression was associated with tumor size, lymph node invasion, differentiation, and TNM stage. However, there was no relationship between IGFBP-3 and IGFBP-3R expression with other parameters including age and sex.

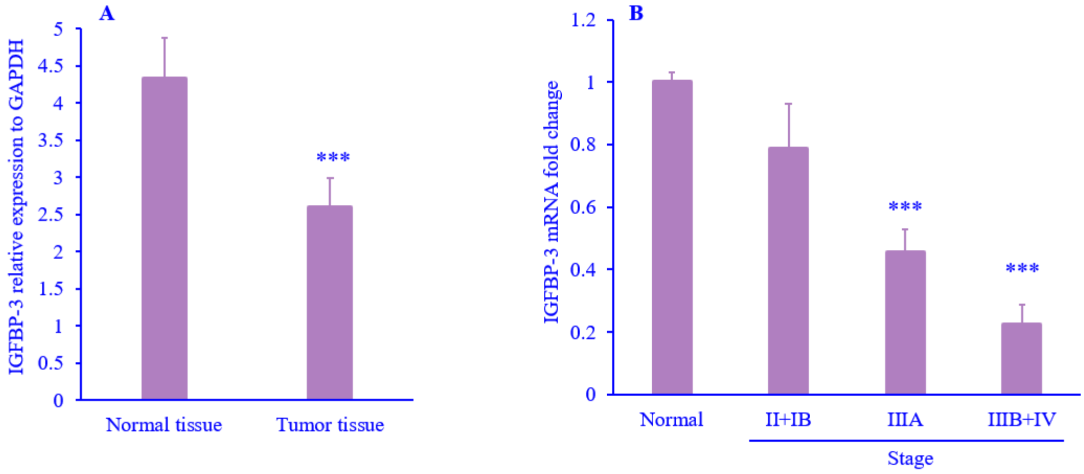

Fig. 1. Down-regulation of the mRNA expression level of IGFBP-3 and tumor-node-metastasis stage analysis in gastric cancer. Relative expression was performed with quantitative real-time polymerase chain reaction and calculated with the $2^{-\Delta\Delta Ct}$ method, normalized all curve thresholds using GAPDH as an internal control. (A) Comparison of relative expression of IGFBP-3 in cancer tissue and normal adjacent tissues; (B) analyzing IGFBP-3 fold-changes relative expression in gastric cancer stages. Data are expressed as mean ± SEM. ****P* < 0.001 represents significant differences in comparison with the control group. IGFBP, Insulin-like growth factor binding protein; GAPDH glyceraldehyde 3-phosphate dehydrogenase.

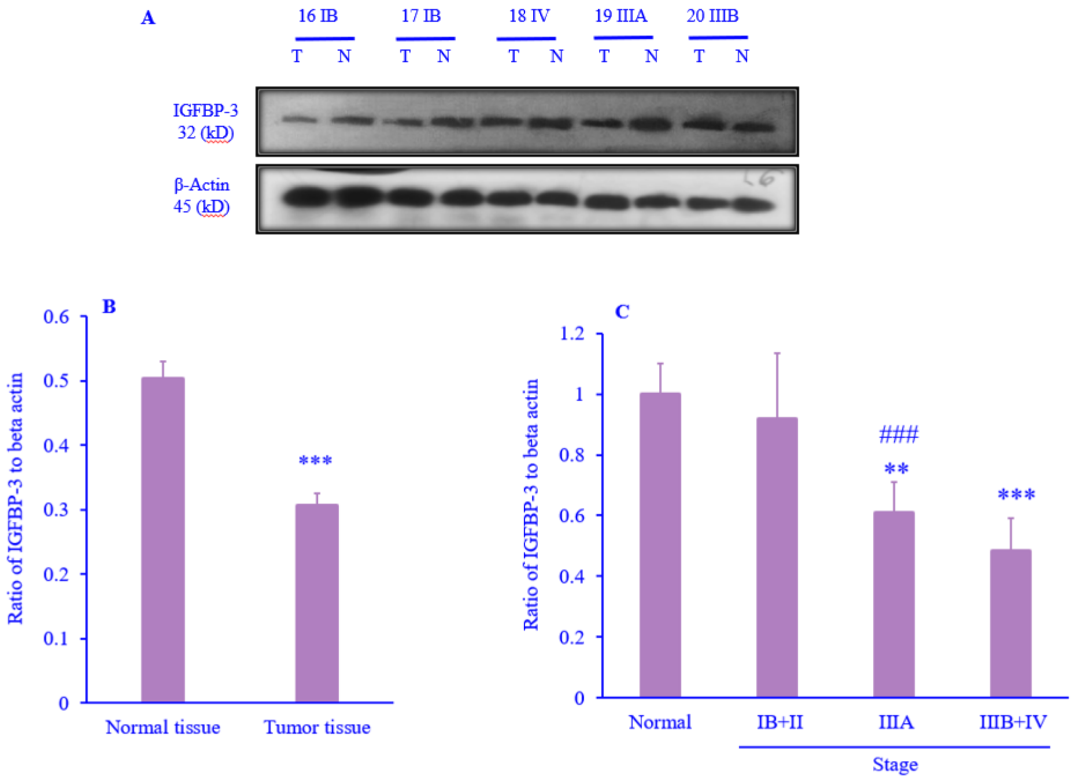

Fig. 2. Protein expression of IGFBP-3 determined with western blotting. All bands were normalized with β-actin as an internal control, and the intensity of all bands was calculated with Image J software. (A) a representative image of IGFBP-3 bands in tumoral cancer and normal adjacent tissue; (B) ratio protein expression in gastric cancer tissue in comparison with normal adjacent tissue; and (C) analysis of protein fold change according to tumor-node-metastasis stage classification. Data are expressed as mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ represent significant differences in comparison with the control group; $^{###}P < 0.001$ indicates differences between a column and its previous one. T, Tumoral cancer; N, normal tissue; IGFBP, insulin-like growth factor binding protein.

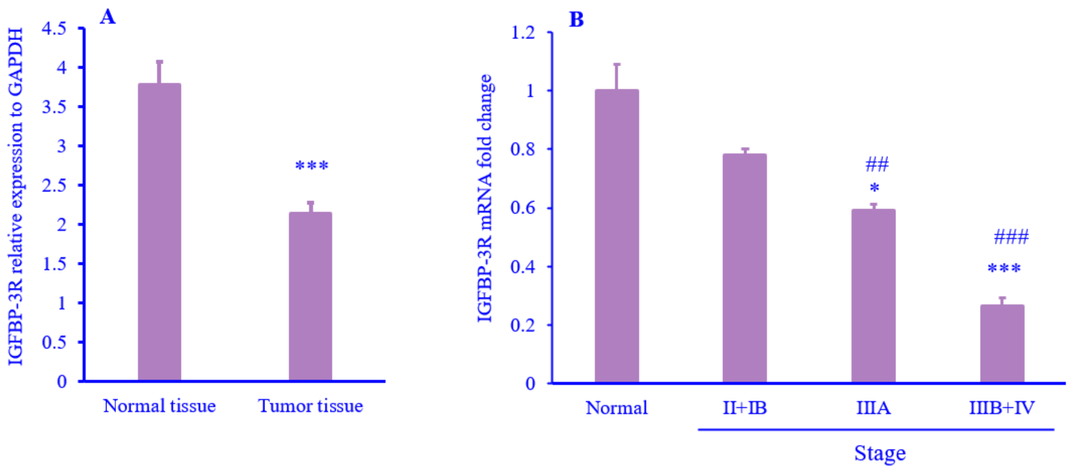

Fig. 3. Analyzing of IGFBP-3R mRNA expression fold change in gastric cancer. (A) Comparison of IGFBP-3R relative expression gastric cancer tissue in comparison with normal adjacent tissue; (B) analyzing IGFBP-3R mRNA fold change in different stages. Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{***}P < 0.001$ represent significant differences in comparison with the control group; $^{##}P < 0.01$ and $^{###}P < 0.001$ indicate differences between a column and its previous one. IGFBP-3R, Insulin-like growth factor binding protein 3 receptor.

Association of IGFBP-3 and its death receptor expression level with OS

The analysis of the association of the OS of the GC patients was performed through Kaplan-Meier with the log-rank test. The low or high expression level was interpreted according to the mean. Patients with high levels of IGFBP-3R mRNA expression (n = 32) had better survival rates than those with low levels of mRNA expression (n = 36) (survival time: 24 ± 1.31 months versus 18 ± 6.33 months, Fig. 5A). However, survival rate analysis based on IGFBP-3 indicated no significant difference between the low and high expression of IGFBP-3 (Fig. 5B).

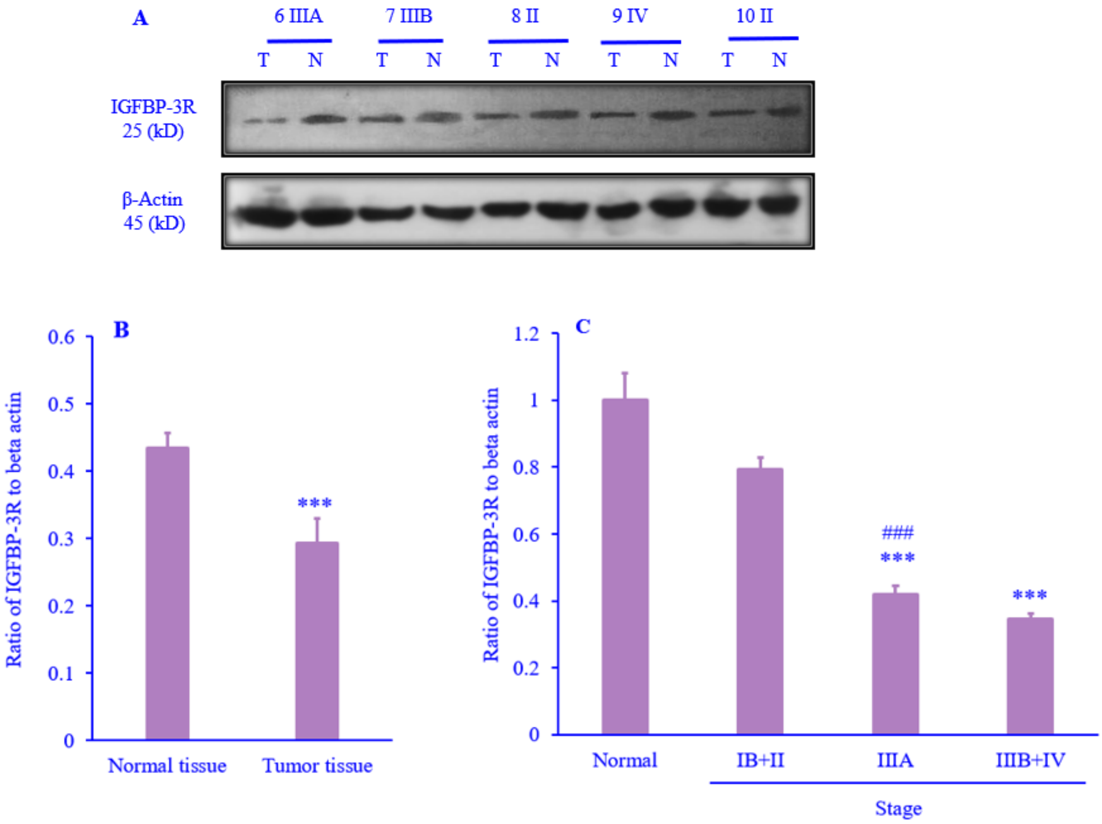

Fig. 4. Analyzing of IGFBP-3R protein expression in gastric cancer and stages by western blotting. All bands normalized with β-actin. (A) The bands indicate the protein expression of IGFBP-3R and β-actin in the tumor and normal tissues; (B) IGFBP-3R protein expression reduced in all patient samples; (C) analysis of IGFBP-3R pattern according to tumor-node-metastasis stage classification. ****P* < 0.001 represents significant differences in comparison with the control group; ###*P* < 0.001 indicates differences between a column and its previous one. T, Tumoral cancer; N, normal tissue; IGFBP-3R, insulin-like growth factor binding protein 3 receptor.

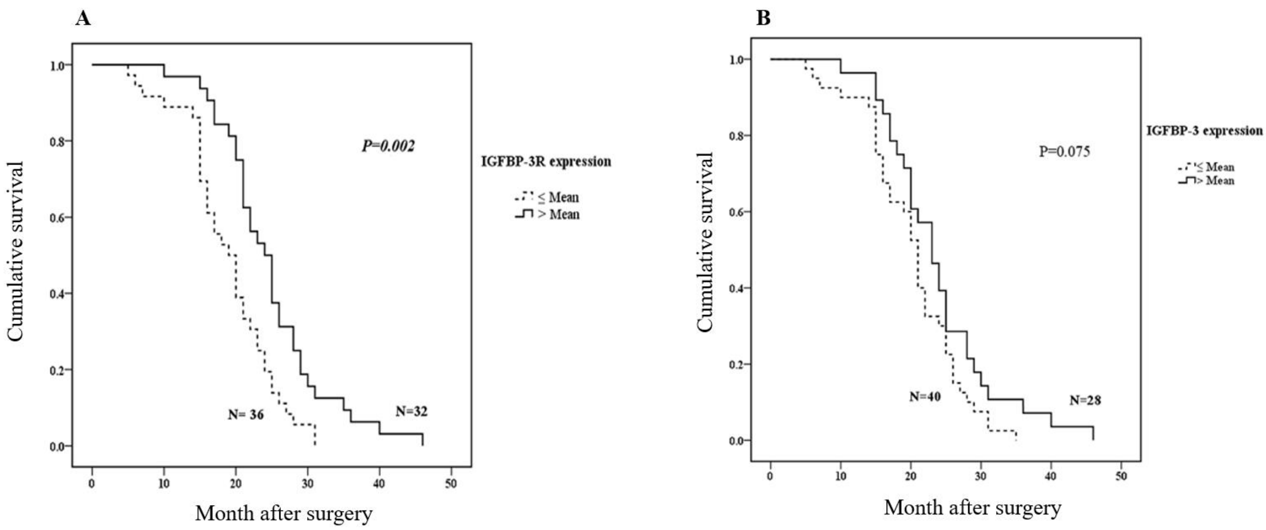

Fig. 5. Correlation of IGFBP-3 and IGFBP-3R expression with cumulative survival patient for 68 gastric cancer patients. Favored or non-favored patient with different expressions (low and high expressions based on mean) was calculated and analyzed with the log-rank test and presented with a Kaplan-Meier plot. (A) The low or high expression level of IGFBP-3R correlates with a poorer overall; (B) relative expression of IGFBP-3 has no significant correlation with the overall survival of a patient with gastric cancer. IGFBP-3, Insulin-like growth factor binding protein 3; IGFBP-3R, insulin-like growth factor binding protein 3 receptor.

DISCUSSION

A proteomics study around the protein biomarkers has indicated that some proteins might serve as potential prognostic biomarkers (15-17). It has been revealed that IGFBPs, specifically IGFBP-3, could be considered a potential prognostic biomarker (18-20). In our previous study, we showed that relative IGFBP-3 expression was reduced stage dependently in the pancreatic adenocarcinoma tumors. In addition, the value for transmembrane protein 219 expression was also reduced in tumors compared to adjacent normal tissues (12). This study, in line with previous studies, was conducted to use IGFBP-3 in the chemosensitization of pancreatic adenocarcinoma tumors (21). Due to the different nature of each cancer, we must know which receptor has high expression and which one has low expression. Therefore, the present study aimed to evaluate IGFBP-3 and its receptor in gastric cancer. Because we have no information about transmembrane protein 219 expression in almost all of cancers, especially GC. The expression of IGFBP3 also appears to be different in various cancers. For instance, Yan *et al.* showed that IGFBP-3 is downregulated in hepatocellular carcinoma (22), but this protein is overexpressed in esophageal squamous cell carcinoma (23).

In the present study, we provided new information about IGFBP-3/IGFBP-3R axis gene expression in mRNA and protein expression levels and analyzed their correlations with clinicopathological features. First, we assessed the differential expression level of IGFBP-3 and its death receptor or transmembrane protein 219.

In the present study, we showed that IGFBP-3 and IGFBP-3R expression were reduced in mRNA levels (Figs. 1A and 3A). In line with our findings, it has been reported that IGFBP-3 promoter methylation, and its reduction of expression, in the early stages of GC are critical in predicting survival (24). Zhang *et al.* also revealed that IGFBP-3 has a protective effect on the development of GC and its downregulation affects the prognosis (25).

The exact mechanism highlighting IGFBP-3 and its receptor roles in GC progression is partially understood (6,7,12,26). In this regard, IGFBP-3 has been extensively considered a p53-inducible gene that initiates apoptosis in cells and tumors (27). Baxter *et al.* also declared that IGFBP-3 is known as a novel ligand mediating apoptosis through nucleus internalization (28,29). Moreover, a previous *in silico* study revealed that IGFBP-3 phosphorylation on serine 111 which occurred in apoptosis induction makes a repulsive effect on IGF-I facilitating IGFBP-3 interaction with IGFBP-3R in the outer membrane (30). Furthermore, Xue and colleagues also indicated that IGFBP-3 can suppress some invasion factors urokinase-type plasminogen activators and matrix metalloproteinase-14 (31). Harada and colleagues also showed that IGFBP-3 can induce apoptosis *via* IGFBP-3R in lung cancer (32). Similarly, IGFBP-3R interacts with the nuclear factor kappa light chain enhancer of activated B cells pathway and suppresses tumor growth (33,34). It was demonstrated that in cancer status, the increasing level of intra- or extra-cellular proteases like matrix metallopeptidases and serine proteases may affect the IGFBP-3 level. Kallikrein 11 in breast cancer can degrade IGFBP-3 and increase the bioactivity of IGFs (35). This fact is supported by our IGFBP-3 western blotting analysis (Fig. 2B and C).

In this study, we also showed that IGFBP-3 was markedly reduced in mRNA and protein levels (Figs. 1A and 2A). This decline correlated with the stage and grade progressions (Table 1, Figs. 1B and 2C). To support IGFBP-3 roles in a higher stage of cancer, a meta-analysis indicated that the reduced IGFBP-3 expression is associated with higher cancer risk, lower survival rate, and more advanced tumor stages of esophageal cancer (36). Similarly, ovarian endometrioid carcinoma, glioblastoma, colorectal cancer, and gastric adenocarcinoma have been reported to be associated with low IGFBP-3 expression (17,37-40). This fact can support our clinicopathological features analysis (Table 1). Consistent with the current study, the low IGFBP-3 expression has been reported to be clinically correlated with higher invasion rates in different cancers including pancreatic ductal adenocarcinoma, ovarian carcinoma, prostate cancer, and non-small cell lung cancer (12,37,41,42).

Similarly, a more recent study uncovered that low expression of IGFBP-3 is linked to certain clinicopathological features and the poor overall survival of patients with hepatocellular carcinoma and pancreatic cancer (12,22). However, in our study, IGFBP-3 expression was not correlated with survival, but IGFBP-3R expression was associated with poor survival (Fig. 5A and B).

CONCLUSION

Taken together, the current study tried to highlight the IGFBP-3/IGFBP-3R axis in GC and represent new information about IGFBP-3R. We demonstrated that a decrease in IGFBP-3 and IGFBP-3R expression is associated with clinicopathological features. Additionally, we demonstrated that IGFBP-3R relative expression was significantly associated with low survival time and poor prognosis in patients with GC. IGFBP-3 and its death receptor expression pattern indicated that they could be recruited as a potential biomarker for TNM staging and prognosis detection. However, further investigations are needed for more validation.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contributions

A. Ansari contributed to writing the original draft, investigation, formal analysis, methodology, and visualization. A. Gheysarzadeh contributed to the investigation, formal analysis, methodology, validation, writing, reviewing, and editing of the article. A. Sharifi analyzed the data, wrote the original draft, edited the article, and also contributed to the investigation, formal analysis, methodology, and visualization. M.R. Mofid contributed to the investigation, methodology, validation, resources, writing, reviewing, and editing of the article, and supervised the study. The finalized article was read and approved by all authors.

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