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Heat Shock Proteins 27, 70 and 90 Protein Expression in Gastric Adenocarcinoma: Prognostic, Predictive and Cellular Differentiation Significances

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Abstract

Background: Gastric cancer is the second global leading cause of death from cancer and the most common gastrointestinal cancer in Iran. This condition is usually diagnosed at advance stages where treatment options are limited. Recently, heat shock proteins (HSPs) have been reported to be overexpressed in a wide range of malignancies and considered as promising candidate biomarkers and therapeutic targets for gastric adenocarcinoma. The aim of the present study was to compare HSPs protein expression between non-tumoral and tumoral sections from patients with gastric adenocarcinoma and determine HSPs protein expression correlation with histological stage, tumoral grade and prognosis.

Methods: Immunohistochemistry was used to assess the expression levels of HSP-27, 70 and -90 proteins on both tumoral and non-tumoral (margin of tumor as control group) sections in 80 patients with gastric adenocarcinoma. Further analyses were histology, grade and stage of tumor (Tumor, node, and metastasis), HSPs expression level, clinicopathological significances, and survival rate.

Results: The expression of HSPs was significantly increased in tumoral sections compared with non-tumoral sections ($P<0.001$). The HSP27 expression was correlated with tumors on the corpus of stomach ($P=0.049$). Patients younger than 63 years revealed higher expression levels of HSP70 ($P=0.040$). High expression levels of HSP90 were further assessed in well-differentiated and intestinal types of tumors ($P=0.009$ and $P=0.019$). Overexpressed levels of HSP27 and 90 were associated with the reduced survival rate of patients ($P=0.017$ and $P=0.018$).

Conclusion: HSP27 and HSP 90 are potential prognostic biomarkers of patients' survival rate. Patients harboring positive HSP27 and HSP 90 expression display worse disease-free survival compared to those with negative HSP27 and HSP 90 expression. Differential expression of HSPs may play crucial roles in the initiation and progression of gastric cancer and can be exploited as future therapeutic targets.

Keywords: Gastric cancer, Heat shock protein, Adenocarcinoma

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Introduction

Heat-shock proteins (HSPs, stress proteins) are a group of evolutionarily conserved chaperone proteins first discovered in 1962.[1] HSPs expression levels increase in stressed cells to protect cells against ischemia, infection, temperature shocks, irradiation, oxidative stress, anticancer chemotherapy, decreased PH, osmotic stress, heavy metals, and other environmental stresses behaving as molecular chaperons for other cellular proteins.[2] HSPs have also been shown to play a critical role in DNA/RNA replication and repair, immune system regulation, apoptosis and cell differentiation.[3-7] HSPs have been classified based on their molecular size: HSP100, HSP90, HSP70, HSP60 and small HSPs (15-30 kDa) such as HSP27. Each family of HSPs is composed of members either expressed constitutively or regulated inductively. These families are targeted to different subcellular parts.[8] HSP90 is constitutively expressed at 2- to 10-fold higher levels in tumor cells compared to their normal counterparts, suggesting that it may be critically important for tumor cell growth and survival.[9] HSP27 may function as a molecular chaperone in signal transduction pathways of different cell regulators and be active in the development of resistance to stressful conditions and agents including cytotoxic drugs.[10] HSP27 may also be a predifferentiation marker.[11] HSP27 and HSP70 have strong cytoprotective properties and their overexpression prevents apoptosis triggered by various stimuli, including hyperthermia, oxidative stress, inhibition of tyrosine kinases by staurosporine, ligation of the Fas/Apo−1/CD95 death receptor or addition of cytotoxic drugs.[12-14] HSPs have been reported to be involved in carcinogenesis in various metabolic and molecular mechanisms such as cell proliferation, invasiveness, neoangiogenesis, metastasization, and induction of immune tolerance.[15-19] Tumor cells that overexpress HSPs may have an increased tendency to invade their microenvironment and spread to distant organs, producing metastasis. For instance, a positive correlation was found between the increased expression of HSP27 and HSP70 and tumor-invasiveness.[20] HSPs may also affect tumor neoangiogenesis; for example, HSP90 stabilizes vascular endothelial growth factor and nitric oxide synthetase in endothelial cells.[21]

Gastric cancer constitutes the fourth most common cancer type and the second most prevalent cause of cancer-related deaths.[22] Although gastric cancer incidence is reduced in Western countries, the frequency of the adeno-carcinomas of the gastroesophageal junction has been soaring.[22] Clinically, the prognosis for advanced gastric cancer is poor, and the 5-year overall survival rates have reached about 15%.[23] Therefore, the elucidation of the molecular pathogenesis of gastric cancer can provide novel targeted therapies resulting in new prognostic and therapeutic avenues. Accordingly, the present work is the immunohistochemical study of the expression levels and clinicopathological significance of HSP27, HSP70, and HSP90 in both tumoral and non-tumoral sections for patients with gastric cancer.

Methods

Patients and Samples

In this case-control study, surgically resected tumoral specimens by total or subtotal gastrectomy were obtained from 80 patients with gastric adenocarcinoma, who had given written informed consent prior to their participation in the study. This study is approved by the Ethics Committee of Mashhad University of Medical Sciences. All 80 patients were admitted to Omid Hospital, Mashhad, Iran. Specimens resected from the non-tumoral margin of tumoral area from the same patients were assumed as the control group. None of the patients received anticancer treatments of any kind (chemotherapy, radiotherapy, or surgical intervention) prior to surgery. Demographic information was registered and tumors were categorized based on Lauren classification as intestinal type and diffuse type. Tumor staging was assessed using the 8th edition of the tumor, node, and metastasis system according to the American Joint Committee on Cancer (AJCC)

system. Pathological investigations revealed three levels of differentiation to classify grading as: well, moderately, and poorly differentiated. 57 patients were followed up for six years, during which time partial and total morbidity was registered. The median survival was 33.2 months.

Immunohistochemistry

Immunostainings for HSP-27-70 and -90 were performed on paraffin-embedded tissue sections using commercially available mouse antihuman HSP-27, HSP-60 and HSP-90 (Santa Cruz Biochemicals, Santa Cruz, CA, USA) primary antibodies. Briefly, 2 μm thick tissue sections

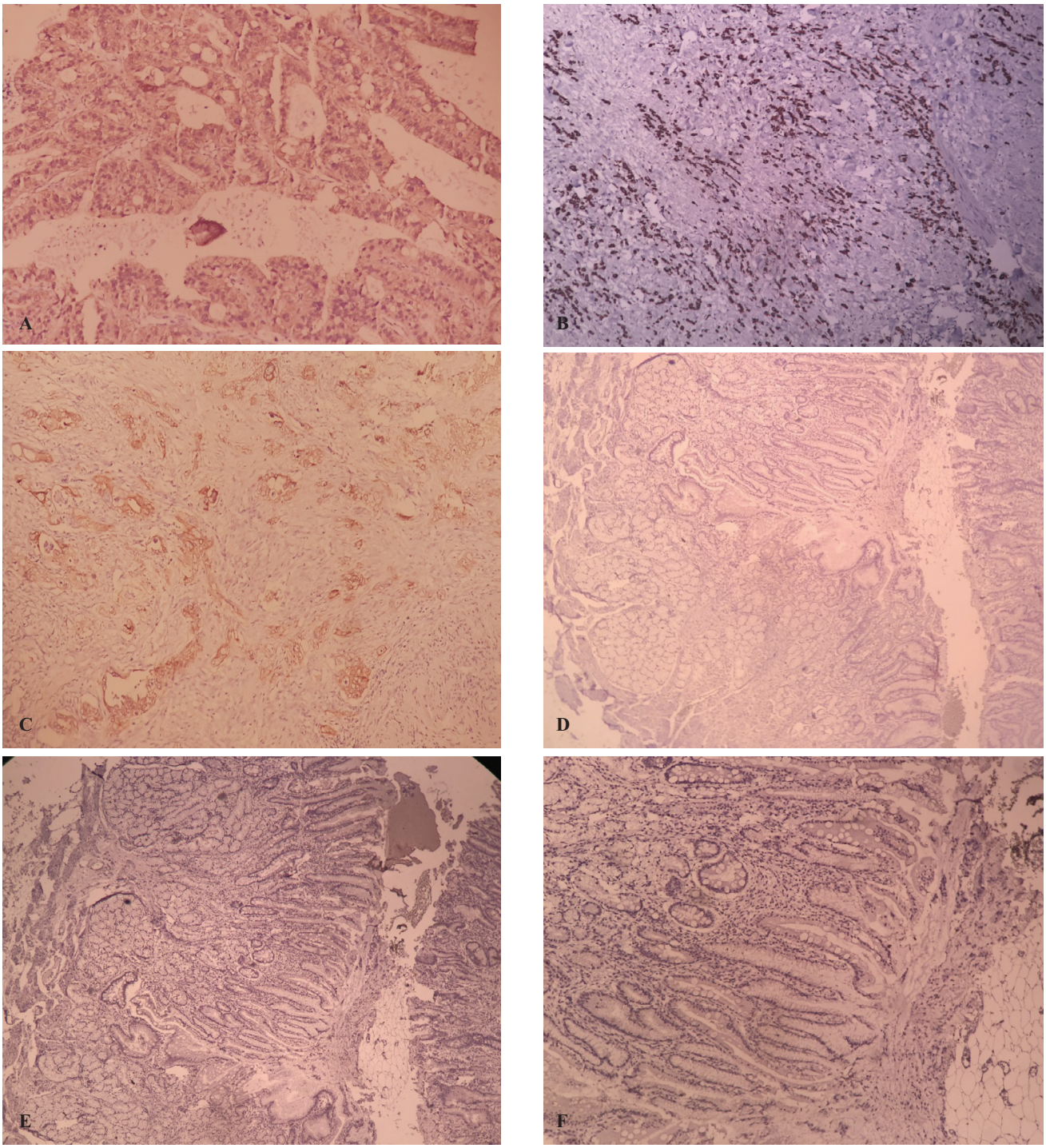

Figure 1. Illustrated HSPs immunostaining in tumor cells with gastric adenocarcinoma: A: HSP-27, B: HSP-70, C: HSP-90 and illustrated HSPs immunostaining in non-tumor cells, D: HSP-27, E: HSP-70, F: HSP-90.

were dewaxed in xylene and rehydrated through graded alcohols. To remove the endogenous peroxidase activity, sections were then treated with freshly prepared 0.3% hydrogen peroxide in methanol in the dark, for 30 minutes at room temperature. HSP-27, -70, and -90 protein expressions were measured using HSP 27 (dilution 1:50, clone 2B4) (Novocastra, Leica Biosystems, Newcastle, United Kingdom), HSP 70 (dilution 1:70, clone 8B11) (Novocastra, Leica Biosystems, Newcastle, United Kingdom), and HSP 90 (dilution 1:100,clone JPB24) (Novocastra, Leica Biosystems, Newcastle, United Kingdom) for 30 min according to manufacturer's protocol. Sections were incubated with Polymer Novolink (Novolink detection system, Leica Biosystems, Newcastle, United Kingdom), stained with Harris' hematoxylin and mounted in Entellan (Merck, Darmstadt, Germany). The percentages of positively stained cells were obtained through counting at least 500 cells in each case, which was done by pathologist observer blinded to the clinical data. The intensity of HSP expression was estimated by high power field light microscope 40X. Specimens were considered "positive" for HSPs when more than 5% of the tumor cells were stained. Only intra-cytoplasmic immunoreactivity was considered as positive.

Statistical Analysis

Data received by demographic, clinical and para-clinical observations were analyzed using SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA). Chi-square was used to assess the association between HSP-27, -70, and -90 overexpression and the intensity of immunostaining and clinicopathological variables. The *P*-value <0.05 was considered (statistically) significant. Spearman rank correlation was used to assess the correlation amongst HSP-27, -70, and -90 protein expressions. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test.

Results

80 patients with gastric adenocarcinoma enrolled in our study, 58 (72.5%) were males and 22 (27.5%) were females. The mean age of the patients was 62.7 ± 8.6 years (median: 62 years, range: 32-82 years). The average age of men was 64.07 years, and the median age in women was 59.32 years. There were no significant differences between men and women's age as indicated by student's t-test (*P*=0.069, t=1.846). 46% of patients were less than 63 years old. Generally, patients with advanced stages of gastric adenocarcinoma (stages 3 and 4) were more than 63 years old. According to Lauren classification, tumors were categorized as 1) intestinal type in 74.7% and 2) diffuse type in 25.3%. Three levels of differentiation were observed, namely well (n=40, 50%),

Table 1. Demographic information regarding the patients in the study

Gender	
Male	72.5%
Female	27.5%
Age	
63<	53.8%
63≥	46.2%
Tumor Site	
Cardia	30%
Corpus – Fundus	37.5%
Antrum	32.5%
Histology of Tumor	
Intestinal	74.7%
Diffuse	25.3%
Tumoral Differentiation	
Well	50%
Moderate	17.5%
Poor	32.5%
T-Score	
T2	3.5%
T3	80%
T4	16.5%
M-Score	
M0	95%
M1	5%
N-Score	
N0	3/16%
N1	50%
N2	2/31%
N3	5/2%
Stage	
1	5/2%
2	8/13%
3	65%
4	8/18%

Table 2. HSP27 expression and intensity of staining and clinicopathological characteristics of the 80 patients with gastric adenocarcinoma

Parameter		HSP-27 expression			*P* –value
		21.5%> n=53	%21.5 ≥ n=27	Total n=80	
Age					
63>		25(31.3)	12(15.0)	37(46.2)	0.053(0.817)
63≤		28(35.0)	15(18.8)	43(53.8)	
Gender					
Male		35(43.8)	23(28.8)	58(72.5)	3.289(0.070)
Female		18(22.5)	4(5.0)	22(27.5)	
Tumoral Differentiation					
Well		25(31.3)	15(18.8)	40(50.0)	0.523(0.770)
Moderate		10(12.5)	4(5.0)	14(17.5)	
Poor		18(22.5)	8(10.0)	26(32.5)	
Tumor Site					
Cardia		17(21.3)	7(8.8)	24(30.0)	7.865(0.049)
Corpus		23(28.8)	6(7.5)	29(36.3)	
Fundus		1(1.3)	0(0.0)	1(1.2)	
Antrum		12(15.0)	14(17.5)	26(32.5)	
PT Classification					
T2		1(1.3)	2(2.5)	3(3.8)	1.427(0.490)
T3		43(53.8)	21(26.3)	64(80.0)	
T4		9(11.3)	4(5.0)	13(16.2)	
PN Classification					
N0	7(8.8)	6(7.5)	13(16.3)	2.813(0.421)	
N1	28(35.0)	12(15.0)	40(50.0)		
N2	16(20.0)	9(11.3)	25(31.2)		
N3	2(2.5)	0(0.0)	2(2.5)		
PM Classification					
M0	37(46.3)	39(48.8)	76(95.0)	0.855(0.355)	
M1	1(1.3)	3(3.8)	4(5.0)		
Histology of Tumor					
Diffused	8(10.7)	11(14.7)	19(25.3)	0.354(0.552)	
Intestinal	28(37.3)	28(37.3)	56(74.7)		
Stage of Tumor					
Stage-I	1(1.3)	1(1.3)	2(2.5)	3.365(0.339)	
Stage-II	6(7.5)	5(6.3)	11(13.8)		
Stage-III	27(33.8)	25(31.3)	52(65.0)		
Stage-IV	4(5.0)	11(13.8)	15(18.8)		

moderately (n=14, 17.5%) and poorly (n=26, 32.5%) differentiated. According to AJCC system, tumor, node, and metastasis staging revealed T2 (n=3, 3.5%), T3 (n=64, 80%), and T4 (n=13, 16.5%). 83.7% of patients were node positive (N1=50%, N2=31.2%, N3=2.5%), while16.3% were node negative (N0). Organ metastasis was noted in 4 out of 80 patients. Clinicopathological

Table 3. HSP70 expression and intensity of staining and clinicopathological characteristics of the 80 patients with gastric adenocarcinoma

Parameter		HSP-70 expression			(*P* –value)
		38.75%> n=38	38.75%≥ n=42	Total n=80	
Age					
	63>	13(16.3)	24(30.0)	37(46.3)	4.220(0.040)
	63≤	25(31.3)	18(22.5)	43(53.8)	
Gender					
	Male	26(32.5)	32(40.0)	58(72.5)	0.604(0.437)
	Female	12(15.0)	10(12.5)	22(27.5)	
Tumoral Differentiation					
	Well	19(23.8)	21(26.3)	40(50.0)	1.200(0.549)
	Moderate	5(6.3)	9(11.3)	14(17.5)	
	Poor	14(17.5)	12(15.0)	26(32.5)	
Tumor Site					
	Cardia	14(17.5)	10(12.5)	24(30.0)	3.562(0.313)
	Corpus	11(13.8)	18(22.5)	29(36.3)	
	Fundus	0(0.0)	1(1.3)	1(1.3)	
	Antrum	13(16.3)	13(16.3)	26(32.5)	
PT Classification					
	T2	1(1.3)	2(2.5)	3(3.8)	2.176(0.337)
	T3	33(41.3)	31(38.8)	64(80.0)	
	T4	4(5.0)	9(11.3)	13(16.3)	
PN Classification					
	N0	7(8.8)	6(7.5)	13(16.3)	3.090(0.378)
	N1	18(22.5)	22(27.5)	40(50.0)	
	N2	13(16.3)	12(15.0)	25(31.3)	
	N3	0(0.0)	2(2.5)	2(2.5)	
PM Classification					
	M0	37(46.3)	39(48.8)	76(95.0)	(0.617)
	M1	1(1.3)	3(3.8)	4(5.0)	
Histology of Tumor					
	Diffused	8(10.7)	11(14.7)	19(25.3)	0.354(0.552)
	Intestinal	28(37.3)	28(37.3)	56(74.7)	
Stage of Tumor					
	Stage-I	1(1.3)	1(1.3)	2(2.5)	3.365(0.339)
	Stage-II	6(7.5)	5(6.3)	11(13.8)	
	Stage-III	27(33.8)	25(31.3)	52(65.0)	
	Stage-IV	4(5.0)	11(13.8)	15(18.8)	

information is summarized in table 1. There was no significant difference between men and woman regarding tumoral differentiation as shown by Chi-square ($\chi 2$=1.044, *P*=0.593). The mean age was 65.08±9.18 in patients with well-differentiated tumors and 58.85±12.16 in poorly differentiated patients. Based on one-way ANOVA test (*P*=0.0556, F=2.985), no statistically significant

Table 4. HSP90 expression and intensity of staining and clinicopathological characteristics of the 80 patients with gastric adenocarcinoma

Parameter		HSP-90 expression			*P* –value
		70.88%> n=27	70.88%≥ n=53	Total n=80	
Age					
	63>	16(20.0)	21(26.3)	37(46.3)	2.775(0.096)
	63≤	11(13.8)	32(40.0)	43(53.8)	
Gender					
	Male	17(21.3)	41(51.3)	58(72.5)	1.859(0.173)
	Female	10(12.5)	12(15.0)	22(27.5)	
Tumoral Differentiation					
	Well	8(10.0)	32(40.0)	40(50.0)	8.103(0.017)
	Moderate	5(6.3)	9(11.3)	14(17.5)	
	Poor	14(17.5)	12(15.0)	26(32.5)	
Tumor Site					
	Cardia	8(10.0)	16(20.0)	24(30.0)	1.153(0.764)
	Corpus	11(13.8)	18(22.5)	29(36.3)	
	Fundus	0(0.0)	1(1.3)	1(1.3)	
	Antrum	8(10.0)	18(22.5)	26(32.5)	
PT Classification					
	T2	0(0.0)	3(3.8)	3(3.8)	3.573(0.168)
	T3	24(30.0)	40(50.0)	64(80.0)	
	T4	3(3.8)	10(12.5)	13(16.3)	
PN Classification					
	N0	4(5.0)	(11.3) 9	13(16.3)	2.082(0.555)
	N1	16(20.0)	(30.0) 24	(50.0) 40	
	N2	6(7.5)	(23.8) 19	(31.3) 25	
	N3	1(1.3)	1(1.3)	2(2.5)	
PM Classification					
	M0	26(32.5)	50(62.5)	76(95.0)	(0.999)
	M1	1(1.3)	3(3.8)	4(5.0)	
Histology of Tumor					
	Diffused	11(14.7)	8(10.7)	19(25.3)	6.908(0.009)
	Intestinal	14(18.7)	42(56.0)	56(74.7)	
Stage of Tumor					
	Stage-I	0(0.0)	2(2.5)	2(2.5)	2.041(0.564)
	Stage-II	3(3.8)	8(10.0)	11(13.8)	
	Stage-III	19(23.8)	33(41.3)	52(65.0)	
	Stage-IV	5(6.3)	10(12.5)	15(18.8)	

difference was observed between the median age of patients and three grade of tumors. Among all 80 patients, 2.5% were in stage I, 13.8% in stage II, 65% in stage III and 18.8 % in stage IV of gastric adenocarcinoma. Likelihood ratio test revealed no significant differences between tumoral stage and age (Likelihood ratio=7.745, *P*=0.060). The tumor site in 30% (n=24) of the patients was in cardia, 36.3% (n=29) in corpus, 1.3% (n=1) in fundus and 32.5% (n=26) in the antrum of

stomach. Likelihood ratio test revealed no significant difference between the tumor site and sex of patients (Likelihood ratio=4.295, *P*=0.249). 38.2% of patients younger than 63 years had histologically diffuse type cancer, while 61.8% had histologically intestinal gastric cancer. A significant difference was found regarding the histology of tumor and age. Diffused tumors were further observed in patients younger than 63 years old ($\chi 2$=5.473, *P*=0.019). Regarding prognosis, there was no correlation between the disease and age, sex, tumor site, tumor histology, and tumor differentiation. However, patients in the advanced stages of the disease (stage 3/4) had lower survival rates compared with patients in the early stages of gastric cancer (*P*=0.034).

HSP-27, -70, and -90 proteins were abundantly expressed in the examined gastric adenocarcinoma cases. Representative immunostainings for HSP-27, -70 and -90 are illustrated in figure 1A–C. In the tumoral sections, 38.8% were positive for HSP27, 60% for HSP70 and 90% for HSP90. None of the non-tumoral sections were positive for HSPs. The mean HSP-27 expression value was 21.5% in tumoral sections, while it was 0% in non-tumoral sections. The expression level of HSP27 was less than the average of HSP27 expression in 53 patients (66.3%) and higher than the average in 27 patients (33.8%). No statistically significant differences were observed between HSP27 protein expression and sex, age, tumoral grade, PT/N/M classification, histology, and stage of tumor (Table 2). There was a significant difference between HSP27 expression and tumor site as determined by likelihood ratio test.

The mean HSP70 expression value was 38.75% in tumoral sections and 0% in non-tumoral sections. HSP70 expression level was less than the median in 38 cases (47.5%) and higher than the average in 42 cases (52.5%). No statistically significant differences were seen between HSP70 protein expression and sex, tumoral grade, PT/N/M classification, histology, and stage of tumor. Likelihood ratio test revealed a significant difference between HSP70 expression value and age (Table3).

The median HSP90 expression value was 70.88% in tumoral sections and 0% in non-tumoral sections. HSP70 expression level was less than the median in 27 subjects (33.8%) and higher than the average in 53 subjects (66.3%). Statistical tests revealed no significant difference between HSP90 expression level and age, sex, tumor site and grade of tumor. Chi-square showed a significant difference between HSP90 expression level and histology and differentiation of tumor (Table 4). We also calculated the Spearman's rank correlation coefficient to evaluate the linear relationships among the expression extents of HSP-27, -70 and -90 proteins. A significant positive correlation was also found between the extent of HSP-60 and HSP-90 expressions. Although a significant positive correlation was observed between the extent of HSP-27, -70 and -90 expressions, the correlation was weak (Table 5). Spearman's rank interpretation was exactly the same as Pearson correlation coefficient. Spearman correlation coefficient was assumed as: $rs < 0.2$ as very weak, $0.2 < rs < 0.4$ as weak, $0.4 < rs < 0.6$ as moderate, $0.6 < rs < 0.8$ as strong, and $rs > 0.8$ as very strong. Among all the 80 patients who were followed up, 17 cases (21.3%) expired. 63 patients were followed up for a median of 60 months (mean 98/4±24/33 months). The mean longevity among cases with HSP27 expression levels lower than average (21.5%) was 37/5±34/28 months, while that of the cases with HSP70 expressions level higher than average (21.5%) was 16/2±50/27 months. Wilcoxon signed-rank test indicated a significant difference regarding the average life span between the two groups of patients ($\chi 2$=5.593, *P*=0.018).The median longevity among cases with HSP70 expression levels lower than average (38.75%) was 1.86±12.08 months, while that of the subjects with HSP70 expression level higher than average (38.75%) was 75/5±57/43 months.

Table 5. Spearman rank correlations amongst the HSP-27, -70 and -90 proteins extent of expression in the 80 gastric tumoral specimens

	HSP-27	HSP-70	HSP-90
HSP-27	-	0/309(0/500)	0/282(0/001)
HSP-70		-	0/309(0/500)

Wilcoxon signed-rank test showed a significant difference in the mean of life span between the two groups ($\chi 2$=5.842, *P*=0.016). The mean life span among cases with HSP90 expression levels lower than average (70.88%) was 56/5±85/25 months, while that of subjects with HSP90 expression levels higher than average (70.88%) was 80/5±65/32 months (Figure 2A–C). Wilcoxon signed-rank test revealed no significant difference regarding the average life span ($\chi 2$=0.001, *P*=0.978). Cox proportional hazard regression showed no statistical significance for the survival rate with age, gender, tumor site, histology of tumor, and grade of the disease.

Discussion

In the present study, the prevalence rate of gastric adenocarcinoma was 2.6 times more in males than females, which is compatible with the investigations of Sadjadi et al. in Iran, Kim et al. in Japan, and Giaginis et al. in Greece.[24-26] In this study, the average age of diagnosis was about 63 years, while it was measured around 55 years by Kim et al.[25] Such discordance may result from the active screening programs for patients in Japan. Only 2.5% of our subjects were diagnosed at stage I and 85% of subjects were diagnosed at stages III and IV of gastric cancer. In the study by Kim et al., 25.6% of the cases were diagnosed at stage I, and 77% were diagnosed at stages III and IV of the disease.[25] This proportion was calculated approximately 53% by Giaginis et al.[26] In a report by Sugasawa et al., less than 70% of the cases were diagnosed at stage II of gastric cancer. According to our results, 87% of the patients were diagnosed with metastasis to lymph nodes, calculated 63% by Giaginis et al., and 55% by Sugasawa et al. In this regard, we can conclude that active screening program for patients can result in diagnosis at the early stage of the disease and more effective therapies. Our results revealed that most patients had well-differentiated tumors (50%). It is concluded that the high expression level of HSPs is associated with the high stage of gastric cancers. The most common tumor sites were located in the stomach, and most tumors (74%) were histologically intestinal. 95% of patients had organ metastasis, 87% were node positive, and no significant difference was observed between the disease stage, differentiation, and tumor site and the gender of the patients. A significant difference existed between the histology of tumor and the age of patients. In this respect, the prevalence rate of the intestinal type of tumor was approximately more than twice the diffused type among patients who were less than 63 years.

HSPs protein expression was dramatically higher in tumoral sections than in non-tumoral sections. None of the non-tumoral sections was positive for any HSPs protein expression although the protein expression was 38.8% for HSP27, 60% for HSP70, and 90% for HSP90 in tumoral sections. In the present study, all examined gastric cancer cases displayed positive expression for at least one member of the HSPs family with variable

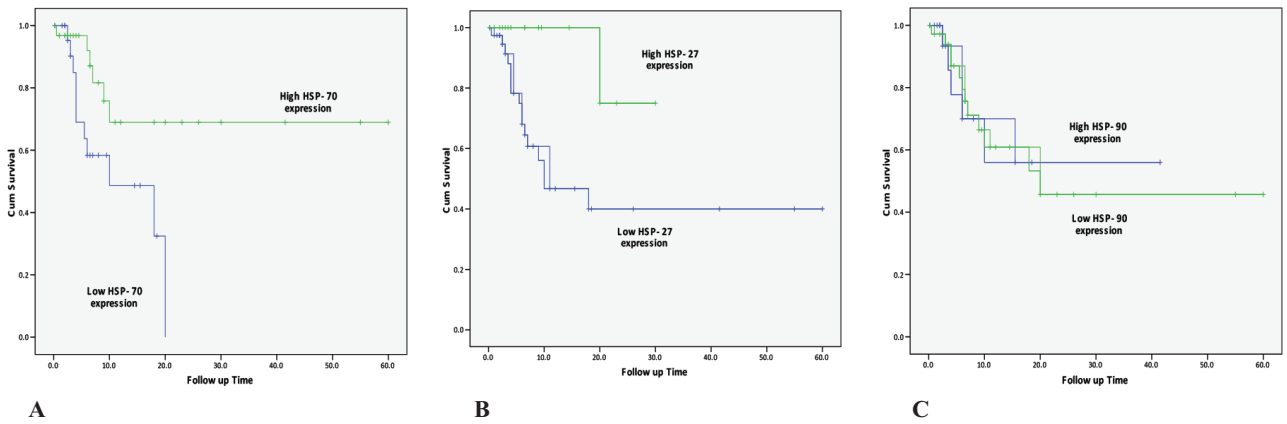

Figure 2. Survival rate in patients with gastric adenocarcinoma during the 5-year follow up according to HSPs protein expression (A. HSP27, B. HSP70, C. HSP90).

expressions in each case. Our results are consistent with earlier reports where HSPs expression was found in bladder cancer and squamous cell carcinoma of the esophagus.[27,28] The present study further supports the clinicopathological significance of HSP-27, -70, and -90 protein expression in the progression of gastric cancer. No association was observed between HSP27 expression and differentiation, histology, and grade of tumor in the present research. On the contrary, previous studies conducted by Nagata et al. (2012), and Bodoor et al. (2016) observed HSP27 expression to be significantly associated with poorly differentiated tumors.[29,30] However, Giaginis et al. reported the relationship between HSP27 and organ metastasis. Kapranos et al. showed an association between HSP27 protein expression and high stages of tumoral sections and female gender.[31] Also, in the present study, we observed an association between high HSP27 expression and tumor site (body of stomach) and low survival rates of patients. A 5-year follow-up up was performed to investigate the patients' survival rate and longevity. The median of patients' life span was 33 months. Kaplan-Meier curve illustrated an association between high HSP27 expression and low survival rates of patients, which is in line with the results of Kapranos et al.[31]

No association has been observed between HSP70 protein expression and gender of patients, disease stage, grade, histology and site of tumor, which is in accordance with Maehara and Isomoto studies.[32,33] One study indicated that this protein exhibited reduced levels of expression in gastric cancer tissues.[29] Nevertheless, certain studies have shown a significant increase in HSP70 expression in gastric cancer sections where >50% of the cases displayed immunoreactivity towards the protein.[33] The hypothesis of the present research was that HSP70 is favorable in the context of transformation and progression as a result of its cytoprotective effects. However, controversial results have been obtained regarding the role of HSP70 in the pathogenesis of gastric cancer. Patients less than 63 years old with low survival rates had high HSP70 protein expressions.

A positive correlation was observed between high HSP90 protein expressions and sex, age and stage of tumor, which is in line with the results of Giaginis et al. High HSP90 expression is associated with well-differentiated and intestinal types of tumors. These findings are consistent with earlier reports investigating the expression of the protein in gastric cancer.[29,34,35] Despite the fact that similar detection methods and scoring criteria were used, we observed the highest expression levels of the protein in GC tissues compared to previous studies. This might be related to the differences in the ethnic backgrounds of patients or different choices of antibodies used in these investigations.

Conclusion

Our study indicated the major role of HSP in many aspects of tumor progression and response to therapy. HSPs have been recently identified as candidate biomarkers and therapeutic targets for a wide range of human malignancies when sufficiently expressed. These proteins were shown to contribute to malignant transformation and progression and to be associated with poor survival. Currently, many efforts are being made (with promising results) to develop antiHSPs compounds for cancer therapy. The current study proposes that the expression of HSP-27, -70 and -90 proteins is associated with clinicopathological significances in terms of diagnosis, prognosis and therapeutic targets for patients with gastric adenocarcinoma. HSP-70 failed to predict patients' prognosis; whereas, HSP-27 and -90 were shown to be promising prognostic indicators in patients with gastric cancer. Further research is required to clarify the molecular pathogenesis through which HSPs' regulation influence the molecular events involved in tumor growth, invasiveness, and metastases. Such studies could be important for the development of new therapeutic strategies, such as using HSP-90 inhibitors and promising prognostic indicators, and targeting HSPs in gastric cancer.

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Conflict of Interest

None declared.

References

1. Ritossa F. A new puffing pattern induced by temperature shock and DNP in drosophila. *Cell Mol Life Sci.* 1962;18(12):571-3.
2. Macario AJ. Heat-shock proteins and molecular chaperones: implications for pathogenesis, diagnostics, and therapeutics. *Int J Clin Lab Res.* 1995;25(2):59-70.
3. Voellmy R. Transduction of the stress signal and mechanisms of transcriptional regulation of heat shock/stress protein gene expression in higher eukaryotes. *Crit Rev Eukaryot Gene Expr.* 1994;4(4):357-401.
4. Macario AJ, Cappello F, Zummo G, Conway de Macario E. Chaperonopathies of senescence and the scrambling of interactions between the chaperoning and the immune systems. *Ann N Y Acad Sci.* 2010;1197:85-93. doi: 10.1111/j.1749-6632.2010.05187.x.
5. Garrido C, Gurbuxani S, Ravagnan L, Kroemer G. Heat shock proteins: endogenous modulators of apoptotic cell death. *Biochem Biophys Res Commun.* 2001;286(3):433-42. doi: 10.1006/bbrc.2001.5427.
6. Luo GR, Chen S, Le WD. Are heat shock proteins therapeutic target for Parkinson's disease? *Int J Biol Sci.* 2006;3(1):20-6. doi: 10.7150/ijbs.3.20.
7. Taylor RP, Benjamin IJ. Small heat shock proteins: a new classification scheme in mammals. *J Mol Cell Cardiol*. 2005;38(3):433-44. doi: 10.1016/j.yjmcc.2004.12.014.
8. Huang Q, Ye J, Huang Q, Chen W, Wang L, Lin W, et al. Heat shock protein 27 is over-expressed in tumor tissues and increased in sera of patients with gastric adenocarcinoma. *Clin Chem Lab Med.* 2010;48(2):263-9. doi: 10.1515/CCLM.2010.043.
9. Isaacs JS, Xu W, Neckers L. Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell.* 2003;3(3):213-7.
10. Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA. Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. *J Natl Cancer Inst.* 1993;85(19):1558-70.
11. Mehlen P, Mehlen A, Godet J, Arrigo AP. hsp27 as a switch between differentiation and apoptosis in murine embryonic stem cells. *J Biol Chem.* 1997;272(50):31657-65 doi: 10.1074/jbc.272.50.31657.
12. Garrido C, Ottavi P, Fromentin A, Hammann A, Arrigo AP, Chauffert B, et al. HSP27 as a mediator of confluence-dependent resistance to cell death induced by anticancer drugs. *Cancer Res.* 1997;57(13):2661-7.
13. Garrido C, Mehlen P, Fromentin A, Hammann A, Assem M, Arrigo AP, et al. Inconstant association between 27-kDa heat-shock protein (Hsp27) content and doxorubicin resistance in human colon cancer cells. The doxorubicin-protecting effect of Hsp27. *Eur J Biochem.* 1996;237(3):653-9. doi: 10.1111/j.1432-1033.1996.0653p.x.
14. Mehlen P, Schulze-Osthoff K, Arrigo AP. Small stress proteins as novel regulators of apoptosis. Heat shock protein 27 blocks Fas/APO-1- and staurosporine-induced cell death. *J Biol Chem.* 1996;271(28):16510-4. doi: 10.1074/jbc.271.28.16510.
15. Czarnecka AM, Campanella C, Zummo G, Cappello F. Mitochondrial chaperones in cancer: from molecular biology to clinical diagnostics. *Cancer Biol Ther.* 2006;5(7):714-20. doi: 10.4161/cbt.5.7.2975.
16. Lemieux P, Oesterreich S, Lawrence J, Steeg P, Hilsenbeck S, Harvey J, et al. The small heat shock protein hsp27 increases invasiveness but decreases motility of breast cancer cells. *Invasion Metastasis.* 1997;17(3):113-23.
17. Sanderson S, Valenti M, Gowan S, Patterson L, Ahmad Z, Workman P, et al. Benzoquinone ansamycin heat shock protein 90 inhibitors modulate multiple functions required for tumor angiogenesis. *Mol Cancer Ther.* 2006;5(3):522-32. doi: 10.1158/1535-7163.MCT-05-0439.
18. Zhao L, Liu L, Wang S, Zhang YF, Yu L, Ding YQ. Differential proteomic analysis of human colorectal carcinoma cell lines metastasis-associated proteins. *J Cancer Res Clin Oncol*. 2007;133(10):771-82. doi: 10.1007/s00432-007-0222-0.
19. Calderwood SK, Theriault JR, Gong J. Message in a bottle: Role of the 70−kDa heat shock protein family in anti−tumor immunity. *Eur J Immunol.* 2005;35(9):2518-27. doi: 10.1002/eji.200535002.
20. Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones.* 2005;10(2):86-103.doi: 10.1379/csc-99r.1.
21. Sun J, Liao JK. Induction of angiogenesis by heat shock protein 90 mediated by protein kinase Akt and endothelial nitric oxide synthase. *Arterioscler Thromb Vasc Biol.* 2004;24(12):2238-44.
22. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin.* 2005;55(2):74-108.
23. Goscinski MA, Larsen SG, Warloe T, Stoldt S, Nesland JM, Suo ZH, et al. Adenocarcinomas on the rise--does it influence survival from oesophageal cancer? *Scand J Surg*. 2009;98(4):214-20. doi: 10.1177/145749690909800404.
24. Sadjadi A, Malekzadeh R, Derakhshan MH, Sepehr A, Nouraie M, Sotoudeh M, et al. Cancer occurrence in Ardabil: Results of a population−based Cancer Registry from Iran. *Int J Cancer.* 2003;107(1):113-8.
25. Kim H, Song K, Park Y, Kang Y, Lee Y, Lee K, et al. Elevated levels of circulating platelet microparticles,

VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. *Eur J Cancer.* 2003;39(2):184-91.
26. Giaginis C, Daskalopoulou SS, Vgenopoulou S, Sfiniadakis I, Kouraklis G, Theocharis SE. Heat Shock Protein-27,-60 and-90 expression in gastric cancer: association with clinicopathological variables and patient survival. *BMC Gastroenterol*. 2009;9:14. doi: 10.1186/1471-230X-9-14.
27. Kawanishi K, Shiozaki H, Doki Y, Sakita I, Inoue M, Yano M, et al. Prognostic significance of heat shock proteins 27 and 70 in patients with squamous cell carcinoma of the esophagus. *Cancer.* 1999;85(8):1649-57.
28. Lebret T, Watson RWG, Molinié V, O'Neill A, Gabriel C, Fitzpatrick JM, et al. Heat shock proteins HSP27, HSP60, HSP70, and HSP90. *Cancer.* 2003;98(5):970-7.
29. Bodoor K, Jalboush S, Matalka I, Abu-Sheikha A, Waq R, Ebwaini H, et al. Heat shock protein association with clinico-pathological characteristics of gastric cancer in jordan: HSP70 is predictive of poor prognosis. Asian Pac *J Cancer Prev.* 2016;17(8):3929-37.
30. Nagata Y, Kudo M, Nagai T, Watanabe T, Kawasaki M, Asakuma Y, et al. Heat shock protein 27 expression is inversely correlated with atrophic gastritis and intraepithelial neoplasia. *Dig Dis Sci.* 2013;58(2):381-8. doi: 10.1007/s10620-012-2342-x.
31. Kapranos N, Kominea A, Konstantinopoulos P, Savva S, Artelaris S, Vandoros G, et al. Expression of the 27-kDa heat shock protein (HSP27) in gastric carcinomas and adjacent normal, metaplastic, and dysplastic gastric mucosa, and its prognostic significance. *J Cancer Res Clin Oncol.* 2002;128(8):426-32. doi: 10.1007/s00432-002-0357-y.
32. Maehara Y, Oki E, Abe T, Tokunaga E, Shibahara K, Kakeji Y, et al. Overexpression of the heat shock protein HSP70 family and p53 protein and prognosis for patients with gastric cancer. *Oncology.* 2000;58(2):144-51. doi: 10.1159/000012091.
33. Isomoto H, Oka M, Yano Y, Kanazawa Y, Soda H, Terada R, et al. Expression of heat shock protein (Hsp) 70 and Hsp 40 in gastric cancer. *Cancer Lett.* 2003;198(2):219-28.
34. Zuo DS, Dai J, Bo AH, Fan J, Xiao XY. Significance of expression of heat shock protein90α in human gastric cancer. *World J Gastroenterol*. 2003;9(11):2616-8.
35. Berezowska S, Novotny A, Bauer K, Feuchtinger A, Slotta-Huspenina J, Becker K, et al. Association between HSP90 and Her2 in gastric and gastroesophageal carcinomas. *PLoS One.* 2013;8(7):e69098. doi: 10.1371/journal.pone.0069098.