Original Research Article

Expression of Bcl-2 and p53 Protein in Stomach Cancer Patients and Its Association with Clinicopathological Factors

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ABSTRACT

Introduction: Stomach cancer is one of the most common cancers worldwide. Bcl-2 and p53 appear to be important biomarkers in patients with stomach cancer. In this study, we investigated the expression of Bcl-2 and p53 proteins and its association with clinicopathological factors in 67 Iranian stomach cancer patients over a 5-year period (2010-2015). **Materials and Methods:** In this cross sectional study, the expression of the Bcl-2 and p53 protein was determined using immunohistochemistry, and the pathological features of tumors were evaluated. **Results:** The expression of Bcl-2 and p53 proteins was detected in 47% and 48% of the patients, respectively. Of the 67 patients, 39 patients had intestinal-type gastric adenocarcinoma, and 57 patients had grade I and II. There was a statistically significant association between Bcl-2 and p53 expressions (P=0.04). **Conclusions:** There is an association between Bcl-2 and p53 protein expressions in patients with stomach cancer. Bcl-2 and p53 protein expressions are more frequent in high-grade tumors. Bcl-2 and p53 expression might play an important role in the early development and phenotypic differentiation of gastric carcinomas, but not so in tumor progression.

KEYWORDS: Stomach cancer, p53 protein, Bcl-2, Expression, Immunohistochemistry

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INTRODUCTION

Stomach cancer is one of the most common cancers and the second cause of cancer deaths worldwide [1]. In Iran, gastrointestinal cancer is prevalent among men, while breast cancer is the most common cancer in women [2]. According to the Iran's cancer registry, stomach cancer and colorectal cancer are the most common gastrointestinal cancers [3]. There is a large geographic difference in the global distribution of stomach cancer, but it is mainly a disease of developed countries with a Western culture [4].

The mechanism of apoptosis modulation in gastric-intestinal epithelia is very complicated. Several genes, factors, proteins or oncogenes and suppressor genes are involved in the process of apoptosis, including *p53*, *bcl-2*, *myc*, *ras*, *Bax* and the Fasl/Fas system [5].

p53 gene mutation is known to play a major role in the carcinogenesis of colorectal and gastric cancers [6]. Immunohistochemical study of p53 accumulation in gastric carcinomas suggest that the *p53* gene alterations are not rare in gastric carcinomas [7]. One study has shown that that 57% of gastric carcinomas express high levels of p53 protein, and there is a strong association between p53 status of the tumor and patient survival time after diagnosis [8].

Bcl-2 is an anti-apoptotic protein that is thought to be involved in biological processes such as tumorigenesis and tumor development [9]. Abnormal p53 and Bcl-2 expression is an important factor in

biological behavior of gastric carcinoma. Increased Bcl-2 expression in gastric cancer results in resistance of cancer cells against the apoptotic effects of chemical drugs or γ-radiation during therapy [10].

Bcl-2 and *p53* genes deregulations are frequently involved in several types of epithelial malignancies. In this study, we investigated the expression of Bcl-2 and p53 protein and its association with clinicopathological factors in Iranian stomach cancer patients over a 5-year period (2010-2015).

MATERIALS AND METHODS

In this cross-sectional study, the expression of Bcl-2 and p53 proteins was determined in 67 stomach cancer patients who underwent gastrostomy. Pathological features of tumors were examined over a 5-year period (2010-2015) at Golestan laboratory and pathology department of 5th Azar Hospital, affiliated to the Golestan University of Medical Sciences in Gorgan, Iran.

Immunohistochemistry was used to determine the Bcl-2 and p53 expression in stomach cancer paraffin-embedded tissues [11]. After sectioning, the tumor specimens were fixed by formaldehyde (10%), embedded in paraffin, and sliced into 4-pm thick sections. The sections were stained with a monoclonal antibody against Bcl-2 and p53 (Clone DO-7; Dako Cytomation, Glostrup, Denmark). The sections were deparaffinized with xylene and progressively dehydrated in decreasing concentrations of alcohol. Endogenous peroxidase activity was blocked by incubation in hydrogen peroxidase 1% in methanol for 30 minutes. The sections were covered with normal goat serum for 15 minutes to reduce nonspecific staining, and then incubated with a 1:20 dilution of the primary antibody for 2 hours at room temperature. The sections were washed with Tris-buffered saline, incubated with a 1:30 dilution of biotinylated goat anti-mouse immunoglobulin G (Tago, Burlingame, CA) for 30 minutes at room temperature. Then, they were covered with a 1:100 dilution of streptavidin-biotin-peroxidase complex (Dakopatts, Copenhagen, Denmark) for 30 minutes at room temperature. The antibody was localized with 3,3-diaminobenzidinetetra- hydrochloride. The slides were stained with 0.3% methyl green for 30 minutes. Negative control samples were prepared without the primary p53 antiserum. When >10% of nuclear-stained cancer cells were included in the section, the tumor was considered to be p53 positive [12, 13]. Immunoreactivity of Bcl-2 was classified into two groups by observing 1000 tumor cells in areas of the sections: no staining in the tumor cells (-), any staining in the tumor cells' cytoplasm and nuclear membrane (+)[14].

Pathologic features including tumor type, depth of tumor, lymph node status, macroscopic appearance, and histological grade were evaluated.

The quantitative and qualitative data were described as mean ± standard deviation (SD) and frequency (percentage), respectively. Differences in categorical variables between expressions of Bcl-2 and p53 protein groups were analyzed using $\chi 2$ and two-tailed Fisher's exact tests. Statistical significance level was set at $P<0.05$. Stata software (version 11, Stata Corp, College Station, TX, USA) was used for all statistical analyses. The study was approved by the Research Ethics Committee of Golestan University of Medical Sciences (Code: IR.GOUMS.REC.1394.272).

RESULTS

The mean age of patients was 62.1±11.3 years (range 29-85). Of the 67 subjects, 53 (79.1%) were male and all of the patients were in clinical stage III. Majority of the

patients were of Persian ethnicity (52.2%), while the rest were Sistani (26.9%) and Turkmen (20.9%).

The tumors were located in the antrum region (58.2%), cardia (38.8), and fundus (3%). According to histological examination, 41.8% of the patients had diffuse-type gastric cancer based on the Lauren's classification [15]. Most tumors (68.7%) were not larger than 5 cm.

The frequency of Bcl-2 and p53 expression in 67 patients with stomach cancer was estimated to be 47% (n=31) and 48% (n=32), respectively. As shown in Table 1, there was a significant association between Bcl-2 and tumor grade (P=0.001).

Table 1. Characteristics of patients with stomach cancer based on p53 protein expression

Characteristic	**Total** (N=67)	**Presence of p53 protein expression** (N=32)	**Absence of p53 protein expression** (N=35)	**P-value** (absence vs. presence)
Values are n (%)				
Age				
≤ 60 years old	37 (55)	18 (48.6)	19 (51.4)	0. 82
> 60 years old	30 (45)	14 (46.7)	16 (53.3)	
Gender				
Male	53 (79.1)	24 (45.3)	29 (54.7)	0.42
Female	14 (20.9)	8 (57.1)	6 (42.9)	
Tumor Type				
Intestinal	39 (58.2)	18 (46.2)	21 (53.8)	0.75
Diffuse	28 (41.8)	14 (50)	14 (50)	
Depth of Tumor				
T1	9 (13.4)	1 (20)	4 (80)	
T2	17 (25.4)	10 (58.8)	7 (41.2)	0.73
T3	39 (58.2)	20 (51.3)	19 (48.7)	
T4	2 (3)	1 (50)	1 (50)	
Lymph node Status				0.78
N0	22 (45.3)	12 (54.5)	10 (45.5)	
N1	15 (22.4)	9 (60)	6 (40)	
N2	11 (16.4)	4 (36.4)	7 (63.6)	
N3	5 (7.5)	3 (60)	2 (40)	
Macroscopic Appearance				0.84
Ulcerated	32 (47.76)	17 (53.1)	15 (46.9)	
Diffuse Infiltrating	25 (37.31)	11 (44)	14 (56)	
Polypoid	10 (14.9)	4 (40.0)	6 (60.0)	
Histological Grade				
GI	32 (47.8)	8 (25)	24 (75)	**0.001**
GII	25 (37.3)	15(60)	10 (40)	
GIII	10 (14.9)	9 (90)	1 (10)	

In addition, p53 expression was significantly correlated with tumor size (P=0.03) and histological grade (P=0.01). Overexpression of p53 was associated with tumor size of larger than 5 cm and advanced stage (P=0.03).

Lymph node metastasis was absent in 49 patients (73.1%) but present in 18 (26.9%). The majority of tumors (85%) were grade I and II (Table 2).

Table 2. Characteristics of patients with stomach cancer based on Bcl-2 expression

Characteristic	Presence of Bcl-2 protein expression (N=31)	Absence of Bcl-2 protein expression (N=36)	P-value (absence vs. presence)
Values are n (%)			
Age			0. 95
≤ 60 years old	14 (46.7)	16 (53.3)	
> 60 years old	17 (45.9)	20 (54.1)	
Gender			
Male	25 (47.2)	28 (52.8)	0.77
Female	8 (57.1)	6 (42.9)	
Tumor Type			
Intestinal	19 (48.7)	20 (51.3)	0.63
Diffuse	12 (42.9)	16 (57.1)	
Depth of Tumor			
T1	3 (60)	2 (40)	
T2	6 (35.3)	11 (64.7)	0.46
T3	21 (53.8)	18 (46.2)	
T4	1 (20)	4 (80)	
Lymph node Status			
N0	11 (50)	11 (50)	0.29
N1	9 (60)	6 (40)	
N2	4 (36.4)	7 (63.6)	
N3	1 (20)	4 (80)	
Macroscopic Appearance			0.92
Ulcerated	16 (50)	16 (50)	
Diffuse Infiltrating	11 (44)	14 (56)	
Polypoid	4 (40.0)	6 (60.0)	
Histological Grade			
GI	8 (25)	24 (75)	**0.001**
GII	15 (60)	10 (40)	
GIII	8 (80)	2 (10)	

Staining for both Bcl-2 and p53 was present in 67 cases. Of 31 Bcl-2-positive tumors, 34.3% showed p53 overexpression, whereas 65.7% of Bcl-2-negative tumors showed p53 Immunoreactivity. Moreover, Bcl-2 expression was significantly associated with p53 expression (Table 3).

Table 3. The association between Bcl-2 expression and p53 expression in stomach cancer patients

		Bcl-2 protein expression N (%)		P-value
		Negative	Positive	
p53 protein expression N (%)	Negative	23 (65.7)	12 (34.3)	**0.004**
	Positive	13 (40.6)	19 (59.4)	
Total		36 (53.7)	31 (46.3)	

DISCUSSION

Over a 5-year period, we found that Bcl-2 and p53 proteins were expressed in 47% and 48% of patients with stomach cancer, respectively. Immunoreactivity was found in 48% of specimens from 67 Iranian patients with stomach cancer. A number of studies have reported the frequency of p53 alteration in gastric cancer to be between 18% and 58% [16, 17]. According to some studies, it is not known whether the *p53* gene mutation can be considered as a prognostic factor. A study reported that the *p53* gene mutation is a prognostic factor [18], while others reported that it does not affect the prognosis [19]. Some studies in Iran have investigated the mutation frequency of p53 among stomach cancer patients. Previous studies have shown overexpression of mutant p53 in 59-63% of patients with stomach cancer using Immunohistochemistry, while sequencing of amplified DNA samples demonstrated the rate to be 25-49% [20].

Azarhoush et al. found nuclear p53 overexpression in 56% of patients with adenocarcinoma of the gastric cardia and 31.3% of patients with adenocarcinoma of the pyloric antrum. They also reported that overexpression of the *p53* gene was significantly more frequent in adenocarcinoma of the cardia than that of the antrum. There was no difference in the clinicopathologic characteristics of the tumors between p53-positive and p53-negative cases [21].

In our study, Bcl-2 protein was expressed in 47% of patients with stomach cancer. In study of Lauwerset al., 72% of the samples from adenocarcinoma patients showed Bcl-2 staining with immunoreactivity in 75% of the tumors. Bcl-2 reactivity was significantly associated with adenocarcinoma of the intestinal morphotype. In addition, 88% of the tumors showed significant immunoreactivity with one of the diffuse tumors (7%). They found a trend of increasing prevalence for immunoreactivity with higher histologic grades in intestinal-type adenocarcinoma. In addition, they found no correlation between Bcl-2 expression and pT stage, lymph node status, and survival [22].

Li et al. reported that abnormal Bcl-2 expression is an important factor in biological behavior of gastric carcinoma, and can affect apoptosis [23].

In our study, Bcl-2 expression was significantly correlated with p53 expression in patients with stomach cancer. Silvestrini et al. indicated that the predictive role of Bcl-2 expression as a prognostic indicator is strongly associated with p53 protein in lymph node-negative breast cancer patients [24]. Jonathan et al. reported that Bcl-2 and p53 are expressed differently in the ovarian cancer cells [25].

In the present study, Bcl-2 expression was significantly correlated with tumor size and histologic tumor grade. Overexpression of p53 was related with tumors greater than 5 cm in size and advanced stage. We could not find any significant relationship between Bcl-2 or p53 expressions and other clinicpathological factors. A study reported that the *p53* gene mutation is associated with tumor location but not with tumor stage or grade [26].

Lauwers et al. reported that Bcl-2 expression in gastric adenocarcinoma appears to be associated almost exclusively with the intestinal morphotype, and is relatively more prevalent in grade 3 tumors. They found no correlation between Bcl-2 expression and pT stage, lymph node status, and survival [22]. Bcl-2 and p53 proteins expression might play an important role in the early development and phenotypic differentiation of gastric carcinomas, but not so in tumor progression [14].

CONCLUSION

Based on the results, there is an association between Bcl-2 and p53 expressions in patients with stomach cancer. Bcl-2 and p53 expressions are more prevalent in high-grade tumors. The routine evaluation of Bcl-2 and p53 expression levels could be useful in identification of patients with more aggressive disease, and contribute to a better therapeutic approach

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study, and data acquisition, analysis and interpretation. FM drafted the manuscript. All authors were involved in critical revision of the article for important intellectual content. All authors have read and approved the final manuscript.

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