

Evaluation of estrogen receptor expression and its relationship with clinicopathologic findings in gastric cancer

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Abstract

Background: The presence of estrogen receptor alpha has been reported in the cell and tissue levels in gastric cancer; however, its impact on patients' survival remains unclear. The aim of this study was to investigate the expression of estrogen receptor in gastric carcinoma as well as its relationship with the clinicopathologic findings of the patients.

Materials and Methods: The study was performed on 100 endoscopic biopsies of gastric adenocarcinoma for estrogen receptor expression using an immunohistochemical method, and their relationship with the clinicopathologic findings of the patients, such as age, gender, tumor site, size, grade, depth of tumor invasion (T), and lymphatic status (N), were analyzed using independent sample *t*-test and Pearson Chi-square test. A $P < 0.05$ was considered significant in all analyses.

Results: Using an immunohistochemical method on endoscopic biopsies of 74 males and 26 females with the mean age of 63 years, estrogen receptor was found to be positive in 41% of patients. No significant difference was found between estrogen receptor expression and other clinicopathologic findings ($P = 0.75$). There was a significant difference between estrogen receptor (+) and estrogen receptor (–) groups in nodal involvement ($P = 0.001$). The estrogen receptor (+) patients had more number of lymph nodes involved.

Conclusion: This study showed that lymph node involvement has a significant relationship with estrogen receptor expression. However, no significant relationship was found between estrogen receptor expression and other clinicopathologic findings such as age, gender, tumor site in stomach, tumor size, tumor grade, and T-stage.

Key Words: Clinicopathologic findings, estrogen receptor, gastric cancer, immunohistochemical technique

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INTRODUCTION

Gastric carcinoma (GC) had been the most prevalent cause of death in the 20th century and is the second most fatal cancer after lung cancer, with an estimated 875,000 new cases diagnosed per year. Although the global incidence of gastric cancer has decreased dramatically in recent decades, it is the most common cancer in north and northwest Iran. The wide variation

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in incidence across different geographic areas and the higher proportion of cardia cancer are two main characteristics of gastric cancer in Iran. It is estimated that in the last decade, the overall survival of patients with GC has not improved and the 5-year survival rate of this carcinoma is less than 20%.^[1]

Surgery is still the radical treatment for GC; however, in most cases, the disease is diagnosed in the advanced stages, so that in only 30–40% of the cases, palliative and curative surgery is possible.^[2] Chemotherapy and radiotherapy are used as adjuvant treatments for patients with T2, 3N in advanced or inoperable GC.^[3-8]

Following the discovery of estrogen receptor (ER) in the cytoplasm of breast cancer cells by Jensen in 1960, scientists have found the ER in GC cells as well, and they suggested that the cells can be regulated by sexual hormones. In a study that was carried out on 68 specimens of GC in China, it was found that on comparing them with 20 specimens from non-cancerous gastric cells, ER was diagnosed in 21 cases (39.9%) of cancer cells, whereas it was absent in non-cancerous stomach tissue.^[9]

In another study carried out in Ukraine, high levels of ER were detected in specimens from GC. Nevertheless, it was not found in the normal tissue of atrophic gastritis.^[10] In a study conducted in England, it was found that 55.8% of GC specimens treated with tamoxifen had positive ER. It should be noted that ER expression was a separate prognostic factor in GC, whereas tamoxifen therapy had no role in the survival rate of the patients.^[11] In a study carried out on 101 specimens of GC in China, methylation of E-cadherin genes, ER, RB1, and P16 was investigated. Hypermethylation was found in 27.7%, 44.6%, 44.6%, and 30.7% of E-cadherin genes, ER, RB1, and P16, respectively. Moreover, ER methylation was found to be related to age and tumor site.^[12,13]

Due to limited use of therapeutic modalities and high prevalence rate of this disease, in this work, we investigated the expression of ER in GC tissue cells using an immunohistochemical method. In addition, we studied the relationship between the mentioned expression and clinicopathologic findings of the patients.

MATERIALS AND METHODS

The study was performed during 1997–2007 on 100 endoscopic biopsies of gastric adenocarcinoma for ER expression using an immunohistochemical method. The results were analyzed considering the clinicopathologic findings of the patients, such as

age, gender, tumor site, size, grade, depth of tumor invasion (T), and lymphatic status (N).

The process of immunohistochemistry was applied for the 4- μ m cuts of paraffin-embedded tissue blocks as follows: (1) Placing the sections in 37°C oven for 48 h; (2) rinsing in 100% xylol, graded ethanol (100%, 85%, and 75%), and distilled water; (3) rinsing in 10% phosphate-buffered saline (PBS) solution; (4) exposure to 10% H₂O₂ and methanol at a ratio of 1:9 for 30 min; (5) rinsing in PBS; (6) placing in citrate-buffered solution (pH = 6.1) for 14 min in a microwave with power 800 W; (7) rinsing in 10% PBS; (8) adding blocking serum to the slides for 30 min and then drying; (9) separately adding the specific antibodies including ER antibody (Dako, clone ID-5, 1/50 dilution) for 30 min at room temperature; (10) rinsing in PBS; (11) adding broad-spectrum antibody for 30 min; (12) adding horseradish peroxidase (HRP)-streptavidin for 30 min; (13) addition of diaminobenzidine for 10 min; (14) rinsing in 10% PBS; (15) dehydration in distilled water, graded alcohols (75%, 85%, 100%), and xylol; (16) counterstaining with hematoxylin, five dips; and (17) mounting. Then, the slides were evaluated by light microscopy. The data were analyzed using quantitative and qualitative variables which were presented as mean and percentage, respectively. The final results were considered positive if $\geq 10\%$ of tumor cells were stained, and they were considered negative if staining was $< 10\%$. Independent sample *t*-test and Pearson Chi-square test were used for comparisons. A $P < 0.05$ was considered significant in all analyses.

RESULTS

In this study, 100 cases of GC were studied for ER expression. All the specimens were investigated for ER by an immunohistochemical method. The results showed 41% positive ER among 74 males and 26 females with age ranging from 31 to 81 years (mean = 63 years). No significant difference was found between the two groups ($P = 0.75$) [Table 1].

Mean age of the patients was 63 years and approximately 40% of the patients were 60–70 years old. The most common tumor location was lesser curvature (55%), followed by cardia (22%), pylor (12%), greater curvature (8%), and fundus (3%). It was found that most of the patients had Grade 2 tumor (59%). We evaluated the relation between ER expression, and localization, size, and grade of the tumor. However, the results showed no statistically significant results ($P > 0.05$). Using the T-classification, no significant difference was found in ER expression between the groups ($P = 0.342$) [Table 2].

Table 1: Gender distribution of the patients

Results of ER	Male		Female		Total	
	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency
Positive	31	31	10	10	41	41
Negative	43	43	16	16	59	59
Total	74	74	26	26	100	100

Pearson Chi-square:0.093, df=1, $P=0.75$. ER: Estrogen receptor

Moreover, it was found that 76% of the patients had T3 tumor [Table 2].

The average number of lymph nodes was 5 (range: 0–35 nodes). Table 3 shows the number of lymph nodes involved in patients, according to TNM classification (PN1 = 1–6, PN2 = 7–15, PN3 ≥ 15). The lymph node involvement was mainly found in the PN1 group (60% of the cases).

Considering the relation between ER expression and nodal involvement, there was a significant difference between ER (+) and ER (–) groups ($P = 0.001$). It means that the ER (+) patients had more number of involved lymph nodes than ER (–) patients [Table 4]. In addition, it was found that patients having high levels of ER expression showed poor prognosis and lower survival rate.

DISCUSSION

GC was the most common cause of cancer deaths in the 20th century and today, it is the second most fatal cancer after lung cancer. In the last 10 years, the survival rate of GC patients has not improved significantly and the 5-year survival rate of these patients is found to be only 20%.^[1] Surgery is the first treatment of choice of GC. However, in most of the cases, progression of GC disease is so rapid that only in 30–40% of the cases, palliative and curative surgery is possible.^[2] In this regard, many scientists have found ER in some of the GC cells and they suggested that the cells can be regulated by sexual hormones.

In our study, of the 100 specimens that were investigated by the immunohistochemical method, 41 (41%) had positive ER and 59 (59%) had no ER (ER–) [Table 1]. As stated earlier, 74% of the patients were male and 26% were female. No significant relationship was found between ER and gender ($P = 0.75$). While in a study performed in Japan on 107 patients suffering from GC, it was found that ER (+) was more in females.^[12]

Moreover, in our study, no relationship was found between age ($P = 0.093$) and tumor site ($P = 0.78$) with ER expression. However, in a study conducted in China, the above-mentioned factors were seen in

Table 2: Tumor T-classification

T-stage	Frequency	Percentage
1	0	-
2	17	17
3	76	76
4	7	7
Total	100	100

Table 3: Frequency and percentage of lymph node involvement among patients

Lymph node	Frequency	Percentage
≤ 6	60	60
6–15	33	33
≥ 15	7	7
Total	100	100

44.7% of patients and ER expression was found to be correlated with age and tumor site.^[14]

In this study, similar to the findings of other studies, there was no significant relationship between tumor site and ER ($P = 0.097$) (15). Moreover, no significant relationship was found between the tumor grade and T-stage ($P = 0.76$), and ER expression [Table 2]. While in a similar study performed in China, same characteristics with a low degree differentiated tumor were seen.^[15] A significant correlation was found between lymph node involvement and ER expression ($P = 0.001$) [Table 4].

In our study, the patients having high levels of ER expression showed poor prognosis and lower survival rate. It has been shown that ER expression has poor prognosis, whereas based on our study, these characteristics were correlated with lymph node involvement. Therefore, it is recommended that in a separate study, the survival rate of ER (+) GC patients be compared with those having no such findings. In addition, it is suggested that hormone therapy such as tamoxifen be investigated in these patients.

On the basis of the present results compared to those of other similar studies, we find that the relationships between ER expressions and clinicopathologic characteristics in each study are different, and this requires further investigation among patients of a specific geographic area.

Table 4: ER expression and nodal involvement

Results Lymph node	ER positive		ER negative		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
≤6	15	15	45	45	60	60
6-15	20	20	13	13	33	33
≥15	6	6	1	1	7	7
Total	41	41	59	59	100	100

T-test for independent sample: 1.865, P-value=0.001. ER: Estrogen receptor

CONCLUSION

From the results of our study, we can conclude that lymph node involvement has a significant relationship with ER expression. However, no significant correlation was found between ER expression and other clinicopathologic findings such as age, gender, tumor site in stomach, the size of tumor, tumor grade, and T-stage.

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