

Association between HER-2/Neu overexpression in gastric adenocarcinoma and histopathologic features of tumor in gastrectomy samples: a cross-sectional study in Kerman, Iran

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Abstract

Background: In tumoral cells, the HER-2/Neu is an oncogene and overexpression would result in further membranous proteins. The role of this gene is known in lung and breast cancers. This study was undertaken to determine the HER-2/Neu overexpression in gastrectomy samples in patients with gastric cancer and its association with tumor histopathology prognostic factors.

Methods: This cross-sectional study was conducted among 48 samples of gastric cancer under gastrectomy. The slides were prepared to assess HER-2/Neu stained by IHC method (Biogenax Kit and DAOK criteria). Statistical analysis was done using SPSS software version 20.0.

Results: Out of 48 cases, 79.2% were males. The mean age was 59.93 ± 13.95 years and the mean tumor size was 5.5 ± 2.45 cm. Majority of cases were in antrum and pylor (43.8%). Lauren class showed a higher frequency of intestinal type (72.9%). In surgery the majority of cases were found to be in T3 stage (87.5%). Her-2/Neu was positive in 10.4% of cases and it was negative in 89.6% of cases. Despite the higher rate of tumors with further expression of HER-2/Neu in those with a size over 5 cm and with vascular invasion, there was no significant association between Her-2/Neu and other variables ($P > 0.05$).

Conclusion: According to the results of this study, overexpression of HER-2/Neu in gastric cancer is low (10.4%) and there is no significant association with other variables. It is suggested that further similar studies with a larger sample size be carried out to definitely determine the association between this marker and prognostic factors.

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Introduction

Gastric cancer is a common cause of cancer-related deaths worldwide (1). Annually over 930 000 new cases are diagnosed and 700 000 deaths are recorded (2). The annual incidence rate of gastric cancer is 50 000 cases (3). Age at onset of this cancer is usually 30-40 years and its incidence rate increases with increasing age. The maximal prevalence rate is observed in the seventh decade of life in men and at comparatively older ages in women. The mean age of disease in Iran is 50-60 years. Approximately 75 percent of cases with gastric cancer would seek care at a physician's office in advanced stages (3-6). The main cause of gastric cancer is not yet clear and *Helicobacter Pylori* and dietary habits are among proposed etiologies. Majority of tumors are sporadic and few cases are familial with autosomal inheritance. Gastric cancer is a multifactorial disease characterized as environmental and genetic factors with predominance of environmental factors in sporadic cases and genetic factors in familial malignancies (7).

HER family includes four receptors; HER1 (EGFR or ErbB1), HER2 (ErbB2 or HER-2/Neu), HER3 (ErbB3), and HER4 (ErbB4). These are involved in cell proliferation, differentiation, and survival (8). These genes are related to many types of tumors and serve as targets for biological treatments for malignancies in breast, colorectal region, lung, head and neck, stomach, and gastroesophageal junction (9). HER2 is an oncogene in gastric cancer and overexpression would result in increased membranous protein in cancerous cells (7) and may affect cellular proliferation, differentiation, apoptosis, and adhesion (10). HER-2/Neu overexpression in gastric cancer would result in poor outcome, more invasive diseases (11) and shorter survival (10-18).

HER-2/Neu overexpression is assessed in many types of malignancies (19-23). Yan et al. reported overexpression of HER-2/Neu in 9.4% of cases leading to lower rate of total survival (20). In a study in Bulgaria (21), the association of positive HER-2/Neu status with worse prognosis was reported. However, poor prognosis of gastric cancer has already been reported in cases with. Some studies have reported definitive observations (22, 23), but clinical relevance of HER-2/Neu overexpression to gastric cancer remains to be established (23,24). Hence, the aim of this study was to determine the status of HER-2/Neu in patients with gastric cancer and its association with histopathological findings in Afzalipour Hospital in Kerman, Iran.

Material and methods

This cross-sectional study was undertaken among 48 cases under gastrectomy for gastric cancer in Afzalipour Hospital in Kerman, Iran from 2014 to 2017. Data were initially gathered from the cancer registry center in Kerman, Iran to identify the gastric cancer cases and the samples were obtained from a pathology lab. The prepared slides were reassessed by a pathologist for the type of tumor (Lauren classification), histological grade, invasion to peripheral tissues and lymph nodes, vascular invasion, presence of *helicobacter pylori*, intestinal metaplasia, and atrophy. After marking for IHC staining, the required cuttings were prepared from paraffinized blocks. The blocks were stained with IHC method for HER-2/Neu marker with Biogenax kit and were observed with Olympus microscope (CX33 model) with amplification of 400* and 100* to determine the staining grade. All sides were graded according to DAKO criteria from 0 to 3+ (Figure 1).

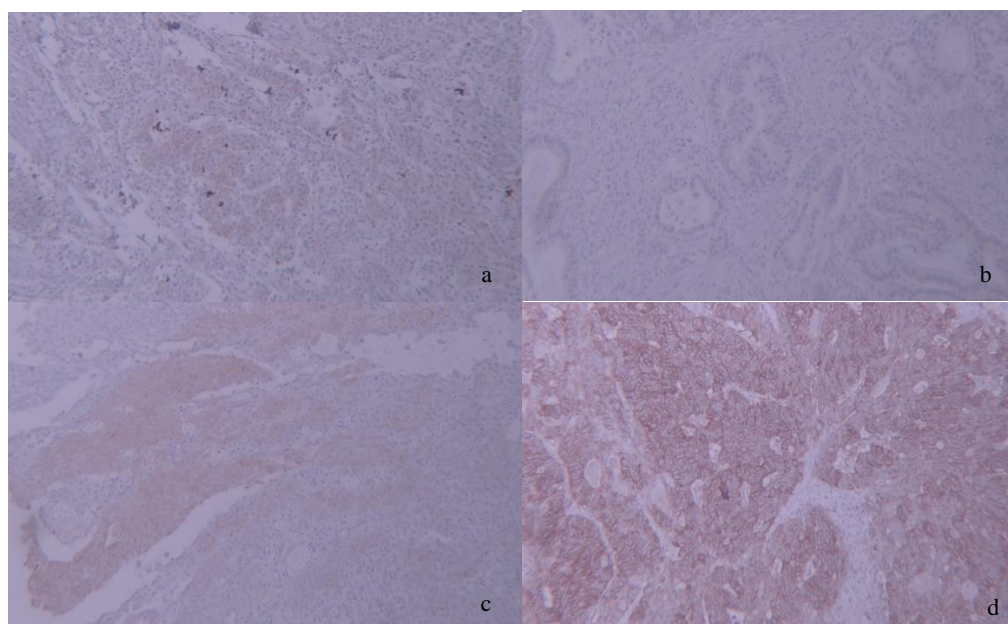


Figure 1. Immunostaining for Her2-neu. a) magnification $\times 100$: negative –grade 0, b) magnification $\times 400$: negative –grade 1+, c) magnification $\times 400$: Weakly positive/equivocal grade +2, d) magnification $\times 400$: Strong positive –grade +3

The DAKO grades for HER-2/Neu include:

0: no membranous staining for HER-2/Neu or less than 10% of tumoral cells are stained as negative expression,

1+: incomplete membranous staining more than 10% of tumoral cells; expression of HER-2/Neu is negative,

2+: complete membranous staining with mild to moderate degree in more than 10% of tumoral cells. These are reported as borderline and should be certified with FISJ/CISH methods.

3+: complete membranous staining in 10% of tumoral cells showing positive HER-2/Neu expression.

Tumor grading was according to American Joint Committee on Cancer: AJCC eighth edition (25) was determined by a pathologist. The invasion grading was categorized into T1, T2, T3, and T4 as follows;

T1; tumoral invasion to lamina propria or muscularis propria or submucosal layer

T2; tumoral invasion to muscularis mucosa

T3; tumoral invasion to subserosa

T4; tumoral invasion to serous layer (visceral peritoneum) or adjacent organs

Tumors were subdivided into good, medium, and poor. However, in this study there were no good cases. The tumor type was as intestinal, diffused, and mixed according to Lauren Classification. (26). Tumor location was in cardia/fundus, corpus, antrum, and pyloric according to Sreeram et al. (27). Data analysis was done using SPSS software version 20.0.

Results

In this study, 48 cases with gastric cancer were enrolled and the HER-2/Neu was positive in 5 cases (10.4%) and negative in 43 samples (89.6%) (Table 1). Thirty eight patients (79.2%) were males and 10 patients (20.8%) were females. The mean age was 59.93 ± 13.95 years (range of 25 to 83). The mean size of gastrectomy samples was 5.5 ± 2.45 cm (range of 1 to 12 cm). The tumor size was larger than 5 cm in 46.6% of cases. Antrum and pyloric regions were involved in 21 cases (43.8%). Majority of cases were intestinal subtype (35 cases,

72.9%). Histological grade of tumor was medium in 56.3%. Majority of cases showed subserosa involvement (87.5%).

The lymphovascular invasion was seen in 91.7% and only 8.3% had no vascular involvement (Table 2).

Table 1. HER-2/Neu expression in gastric samples

Grade	HER-2/Neu Overexpression	Frequency	Total Count
0	Negative	32	43 (89.6%)
1+	Negative	9	
2+	Negative (without molecular method)	2	
3+	Positive	5	5 (10.4%)
Total Count			48 (100%)

HER-2/Neu overexpression was seen in 18.2% of subjects older than 70 years of age but it was completely negative in those aging under 50 years (P=0.814). This was observed in 10.5% and 10% of male and female subjects (P=0.961). HER-2/Neu was positive in 17.6% of tumors in cardia/fundus regions

and the other regions were completely negative (P=0.545). In 16.1% of tumors larger than 5 cm the HER-2/Neu was positive (P=0.382). There was no significant association between HER-2/Neu and other variables (P > 0.05).

Table 2. Histopathological variables and their association with HER-2/Neu

Variables	Frequency (%)	HER-2 positive cases	P value
Age (Year)			
<30	1 (2.1)	0	0.814
31-40	4 (8.3)	0	
41-50	7 (14.6)	0	
51-60	10 (20.18)	1 (10)	
61-70	15 (31.3)	2 (13.3)	
70<	11 (22.9)	2 (18.2)	
Sex			
Male	38 (79.2)	4 (10.5)	0.961
Female	10 (20.8)	1 (10)	
Tumor Location			
Anthrum/Pylor	21 (43.8)	2 (9.5)	0.545
Anthrum/Body	6 (12.5)	•	
Cardia/Fundus	17 (35.4)	3 (17.6)	
Corpus	4 (8.3)	•	
Tumor Size (cm)			
<5	13 (27.1)	•	0.382
≥5	31 (46.6)	5 (16.1)	
Diffused (not sizable)	4 (8.4)	•	
Lauren Class			
Intestinal	35 (72.9)	3 (8.6)	0.689
Diffused	12 (25)	2 (16.7)	
Mixed	1 (2.1)	•	
Histological Grade			
Medium	27 (56.3)	3 (11.1)	0.858
Poor	21 (43.8)	2 (9.5)	
T Stage			
T1	4 (8.3)	1 (25)	0.758
T2	1 (2.1)	•	
T3	42 (87.5)	4 (9.5)	
T4	1 (2.1)	•	
Lymphovascular Invasion			
Positive	44 (91.7)	5 (11.4)	0.476
Negative	4 (8.3)	•	

Discussion

Gastric cancer is the most common gastrointestinal malignancy (28). Majority of subjects in northern and north-western Iran are at increased rate (3). The discovery of certain genes, such as HER-2/Neu and P53 indicate that the clinical course and prognosis in patients with gastric cancer may be predicted better (29). Type of HER-2/Neu expression may be related to prognosis, lymph node metastasis, distant metastasis, survival, and therapeutic response (30). But the association of HER2-Neu and histopathological criteria in many studies are different.

This study assessed HER-2/Neu overexpression in 48 cases with gastric cancer and the association with histological findings was investigated. In congruence with the study conducted by Gurel et al. (31), HER-2/Neu was positive in 10.4% of cases that is lower than other studies (21,32,33). In the studies of Barros-Silva et al. in Portugal and Grabsch et al. in Germany and the United Kingdom, HER-2/Neu test was positive in less than 10 percent of participants (34,35). In some local studies also a higher positive rate was reported for HER-2/Neu. This rate was 24.6% and 15.4% in the studies conducted by Janbabaee et al. and Emadian et al. respectively (30,36). This may be due to genetic and biological differences across the regions. In the current study, HER-2/Neu was positive in subjects older than 50 years. But there was no association with age. This was similar to other studies (37-39). Hence, age is not related to HER-2/Neu expression. HER-2/Neu was comparatively more common in men but there was no significant association between gender and this gene in the study of Oshima et al. (37).

Positive HER-2/Neu was seen in tumors larger than 5 cm. But there was no significant association between the tumor size

and HER-2/Neu overexpression in the study of Park et al (13). Conversely, significant association was observed in the studies conducted by Shi et al. (32) and Janbabaee et al. (30). This may be related to more positive cases of HER-2/Neu and a larger sample size in their studies. In some studies, the majority of cases with positive HER-2/Neu were in the first stage of disease, with no significant association with the pathological stage (31,37). The research study conducted by Abbasi et al. (40) showed higher HER-2/Neu overexpression in patients with gastric cancers in higher stages.

HER-2/Neu expression was higher in cases with medium grades compared with poor grades. But there was no association as well as other studies (31,37). Ognjenovic et al. (41) reported significant association between HER-2/Neu expression and tumor differentiation. Cardia and fundus had higher expression of HER-2/Neu without significant association, but Xu et al. (39) and Ognjenovic et al. (41) reported significant associations.

HER-2/Neu expression was higher in diffuse type of gastric cancer. But there was no significant association with Lauren classification. On the contrary, the study conducted by Emadian et al. reported a significant association (36). Park et al. (13) reported that intestinal cases had significantly a higher rate of HER-2/Neu compared with the diffused type. The authors declaimed that the possible explanations are complex and would require further assessment. Association of this oncogene with especial tumor subtype shows that some exclusive characteristics may be seen simultaneously (13).

Conclusion

The findings in the current study revealed that HER-2/Neu is positive in 10.4% of cases. There was no significant

association between HER-2/Neu overexpression and understudy variables. It is suggested that further similar studies with a larger sample size be carried out to definitely determine the association between this marker and prognostic factors.

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