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HER-2/neu Expression in Resectable Gastric Cancer and its Relationship with Histopathologic Subtype, Grade, and Stage

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Abstract

Objective
HER-2/neu is overexpressed in diverse human cancers. Studies suggest a role of this protein in tumor progression by specifically promoting the invasive capacity of tumor cells. Our aim was to evaluate HER-2/neu overexpression in resectable gastric cancer in 100 North-Eastern Iranian patients and to assess the relationship between its expression and clinicopathologic tumor parameters.
Materials and Methods
Indirect immunostaining was employed to evaluate the expression of this receptor in formalin-fixed paraffin-embedded tissue samples.
Results
HER-2/neu overexpression was present in 26 (26%) of 100 gastric carcinomas. This was significantly more common in the intestinal type of gastric cancer (33%) compared to diffuse (5%) or the mixed type (0%). HER-2/neu overexpression was also more common in well-differentiated gastric cancers versus other grades (41% vs 7%). However, it was not associated with gender, age at diagnosis or stage.
Conclusion
HER-2/neu overexpression is common in gastric carcinoma and more prevalent in intestinal and well-differentiated subtypes. There is no correlation between HER-2/neu expression and tumor stage. The relatively high percentage of HER-2/neu positive tumors may provide a useful target for immunotherapy of these cancers.

Keywords: HER-2/neu, Gastric cancer, Grade, Immunohistochemistry, Lauren's classification.

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Introduction

HER-2/neu oncogene, also known as c-erbB-2, encodes a transmembrane tyrosine kinase receptor, homologous to epidermal growth factor receptor (1). HER molecules belong to a family of glycoproteins that consist of an extracellular domain for binding ligands, a short lipophilic transmembrane domain, and an intracellular domain carrying tyrosine kinase activity. HER-2/neu gene, located on chromosome 17q21, is related to the oncogene v-erbB of the avian erythroblasosis virus. The protein encoded by this gene – p185 – is suggested to be a growth factor receptor involved in the growth and progression of malignant cells. However, the ligand of p185 is not yet identified (2).

HER-2/neu gene is found to be amplifiend in 10% to 30% of human breast, ovarian, gastric and other diverse cancers including lung adenocarcinoma, uterine cervix carcinoma and squamous cell carcinomas of the head and neck (3-6). Depending on the methodological aspects of the assays, scoring criteria and case selection, the prevalence of HER-2/neu expression shows wide variation in different studies on various tumors.

The prognostic value of c-erbB-2 has been primarily shown in breast cancer where patients with overexpression of this gene have a significantly lower relapse free and overall survival rate than patients without overexpression (7-12).

Evidence also exists in gastric cancer showing that overexpression of this protein is a new, independent prognostic factor for overall survival (13). However, some studies have failed to find an association with prognosis (14-17). On the other hand, with the availability of the monoclonal antibody trastuzumab, HER-2/neu can be the target of therapy in this disease, adding to the importance of research on HER-2. (18).

Immunohistochemistry and fluorescence in situ hybridization (FISH) are the techniques routinely recommended for determining HER-2/neu status, in terms of protein overexpression and genomic amplification, respectively (18).

The aims of this study are: 1) to assess HER-2/neu content in our gastric carcinoma patients and 2) to assess the correlation between this receptor tumor content and clinicopathologic characteristics.

Patients and Methods

One hundred gastric adenocarcinoma pathologic specimens from patients who had undergone curative surgery between 2000 and 2006 were identified from the specimen archive, Pathology Department, Omid University Hospital, Mashhad, Iran. All patients had been residing in North – Eastern Provinces of Iran at the time of surgery. TNM staging had been performed on all patients according to AJCC (American Joint Committee on Cancer) staging before treatment. No patient had received chemotherapy or radiation therapy before curative resection. All patients had undergone staging procedures by supra- and infra- diaphragmatic imaging studies, i.e. chest X-ray and abdominal ultrasonography or CT scanning.

Immunohistochemistry

A section from specimen blocks was stained with H&E for histological subtype evaluation and the grade was determined. Representative blocks were chosen for immunostaining. Four-micrometer-thick sections were dewaxed and processed for immunohistochemistry. IHC was performed with the HercepTest kit (k5204, Dakocytomation, Denmark) according to manufacture's instructions.

Diaminobenzidine was used as the chromogen, and the slides were counterstained with hematoxylin. We used known positive breast cancer cases as

positive control. For negative control we omitted the primary antibody.

All sections were evaluated by our pathologist without knowledge of the clinical characteristics of the patients. Tumors with more than 10% of cancer cells showing membranous staining for HER-2 were classified as positive. Cytoplasmic staining was not accounted. The results were reported according to the Dako manual for interpretation of HER-2 staining, a semiquantitative system based on intensity of cell-membrane immunostaining and the percentage of positive cells in the suitable carcinoma fields (score range from 0 to 3+). In this study, as in breast cancer, scores 2 and 3 of IHC intensity were assumed to be positive for overexpression in any percentage of cells (Figures 1-3).

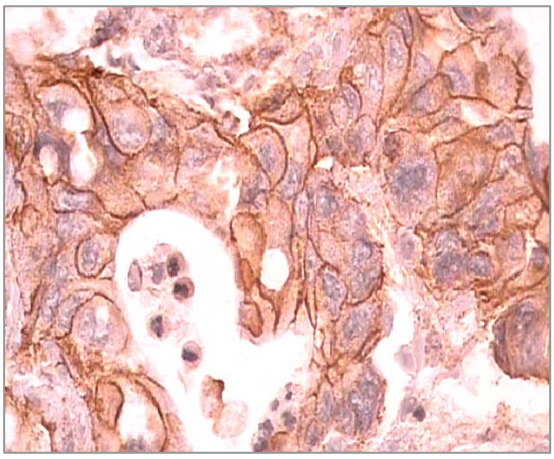

Figure 1. HER-2/neu scores 3+ overexpression. (40x magnification)

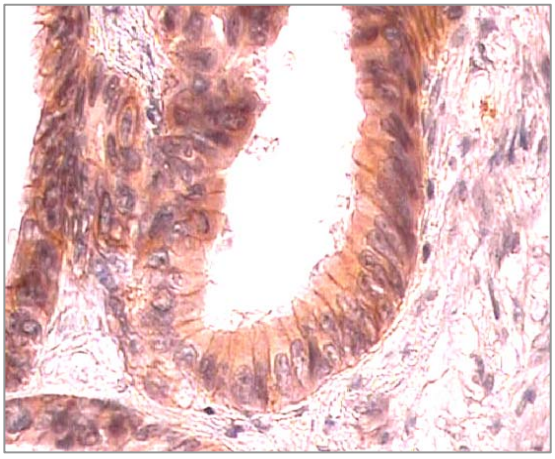

Figure 2. HER-2/neu scores 2+ overexpression. (40x magnification)

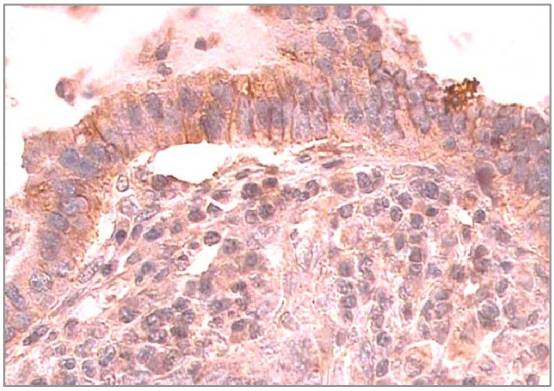

Figure 3. HER-2/neu score1+ overexpression. (40x magnification)

The relation between the HER-2/neu staining and clinicopathologic variables was analyzed. SPSS 11.5 was used for statistical analysis. p values were calculated by using Chi-square, Fisher exact test, t test and ANOVA.

Results

The mean age was 63.31 years (from 26 to 82) and the male/female ratio was 2.8 (74 men, 26 women). 3% of cases were stage I, 13% stage II, 64% stage III, 20% stage IV. According to Lauren's classification, 74 tumors (74%) were intestinal, 21 (21%) were diffuse and five (5%) were mixed-type carcinomas. 54 tumors were well differentiated, (Grade 1), 17 were moderately-differentiated (Grade 2) and 29 were poorly-differentiated (Grade 3). From 100 tumors, 36 were located at the cardia, one at the fundus, 33 at the corpus and 30 in the antrum. The positive staining with IHC method ranged from 0 to 90 percent of tumoral cells. In 26 out of 100 samples (26%) HER-2/neu protein overexpression was observed. Positive reactivity with anti c-

erbB-2 antibody was significantly more frequent in low grade (differentiated) tumors rather than high grade (undifferentiated) tumors (p=0.001) (Table 1).

Table 1. Relationship between HER-2/neu expression and clinicopathologic features in patients with gastric cancer

Cilnicopathologic data	n	HER-2/neu		pvalue
		pos	%	
Sex				Not Significant
Male	74	20	27%	
female	26	6	23%	
Age				
>65	53	15	28%	Not Significant
<65	47	11	23%	
T				
T1	0	0	-	
T2	4	1	25%	Not Significant
T3	79	21	26%	
T4	17	4	23%	
N				
N0	16	5	31%	
N1	51	16	31%	Not Significant
N2	30	4	13%	
N3	3	1	33%	
Location of tumor				
Cardia	36	10	27%	
Fundus	1	0	-	Not Significant
Body	33	9	27%	
Antrum	30	7	23%	
Histology				
Intestinal	74	25	33%	p=0.001
Diffuse	21	1	5%	
Mixed	5	0	-	
Differentation				
Well-diff	54	22	41%	
Mod-diff	17	2	11%	p=0.001
Poorly-diff	29	2	7%	
Stage of cancer				
1	3	0	-	
2	13	6	46%	Not Significant
3	64	16	25%	
4	20	4	20%	
M				
M0	96	26	27%	Not Significant
M1	4	0	--	

n: number, pos: positive, T: Tumot, N: Node, M: Metastasis

Statistical Analysis

A significantly higher expression of c-erbB-2 was observed in tumors with intestinal histology compared to diffuse or mixed types (33% vs 5% vs 0%, p=0.001). With the exception of tumor grade and tumor histology, no other significant correlations were observed between overexpression of this protein and studied variables (Table1). The mean age in patients with poorly

differentiated tumors was lower than other patients (p=0.019). Besides, the mean age in patients with HER-2/neu positive tumors was lower than patients with negative HER-2/neu (p=0.001). Stage IV disease was significantly more common in patients younger than 65 years and all stage I cases were observed among patients older than 65. The number of patients with diffuse histology was two times higher in patients under 65 yrs old.

Discussion

Tumors of the upper gastrointestinal tract have been reported to show a wide range of over expression of HER-2/neu protein (19). In the present study we investigated the expression of this receptor protein in 100 Iranian gastric cancer patients, retrospectively. Our observed prevalence of HER-2/neu protein over expression by immunohistochemistry (26%) fits the range previously reported (8.2-27.5%) (20). However, at the upper range there are some limitations inherent in IHC technique, such as those caused by variability in fixation, and the problems in standardization and scoring of the staining. In a FISH-based study recently reported, HER-2/neu was found to be amplified in about (18%) of the cases (21). Our percentage can be modified as well if we employ FISH method – the state-of-the-art alternative to IHC – on the same samples. This is designed as the future direction of this project.

As in breast carcinoma, where HER-2/neu gene amplification is common in invasive ductal carcinoma and an uncommon feature in lobular carcinoma, the intestinal type of gastric cancer shows higher prevalence of HER-2/neu amplification in contrast to diffuse type (21). Our results also confirm that HER-2/neu protein over expression is strongly associated with the intestinal histological subtype as defined according to Lauren's classification (33% in intestinal versus 5% in diffuse subtype, P= 0.001). In other three studies (22-24), immunohistochemical HER-2/neu overexpression has been as well more common in the intestinal rather than diffuse type of gastric adenocarcinoma. However, so far it has been difficult to evaluate the clinical importance of this finding.

In this study, well differentiated tumors were more likely to overexpress the HER-2/neu protein. This is in agreement with the results reported by other investigators (25, 26). HER-2/neu gene amplification has been associated with the degree of differentiation of adenocarcinomas, in that well differentiated adenocarcinomas have shown a very high incidence (up to 100%) of HER-2/neu amplification (27, 28). However, not all studies agree, and a positive relation with poor tumor differentiation has been reported as well (29).

In this work, regarding previous published studies (19, 30-32), we considered HER-2/neu over expression as a prognostic factor, depicting a poor prognosis. On the other hand, surgical stage is by itself another proven prognostic factor. Our aim was to establish a correlation between HER-2/neu expression status and stage. The absence of this relation in our results might be due to the fact that HER-2/neu positive tumors have a more aggressive pathologic behavior, which is reflected in more common micrometastatic disease at presentation and more distant failures later after treatment. Additionally, it should be noted that there are inconsistencies in the literature regarding the clinical significance of HER-2 over expression which may be due to problems of selecting the best methodology, standardizing the methods, and determining the correct cut off points.

The present drug sensitivity assays suggest that the presence of HER-2/neu amplification may turn out to be a more important feature in therapeutic prediction than in prognostication in gastric adenocarcinomas (21). Analysis of a HER-2/neu amplified gastric carcinoma cell line N87 indicated that

it was as sensitive to trastuzumab as the HER-2/neu amplified breast carcinoma cell line SKBR-3, which is a widely used reference in trastuzumab sensitivity studies (33, 34).This will open the doors to new treatment approaches in gastric cancer in future.

Future Direction

Regarding the high prevalence of gastric cancer in this country and its poor outcome, the importance of research on novel prognostic and therapeutic measures can not be overlooked. With increasing the sample size in future studies and designing prospective studies with close observation of survival, we will be trying to determine the prognostic role of HER-2/neu over expression. Besides, FISH or CISH methods will be utilized to detect the gene amplification.

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