Immunohistochemical expression of HER-2/neu receptors in gastric carcinoma

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Background/aim
Gastric carcinoma is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related death. It is associated with patient morbidity and mortality worldwide. Adenocarcinomas represent 90% of gastric carcinoma cases and are classified into intestinal and diffuse types. Human epidermal growth factor receptor 2 (HER-2)/neu overexpression is a predictor of poor prognosis. This study aimed to evaluate HER-2/neu expression in gastric carcinomas and to correlate its expression with clinicopathological parameters and other prognostic factors.

Patients and methods
Forty-five cases of primary gastric carcinoma were included in this study. Thirty-two cases were of intestinal type and 13 cases were of the diffuse type. All specimens were formalin fixed, routinely processed, stained with hematoxylin and eosin, and immunohistochemically stained with HER-2/neu.

Results
Overexpression of HER-2/neu was detected in five out of 30 (11.1%) gastric carcinomas. There was a nonsignificant difference ($P>0.05$) in different histologic types. Also HER-2/neu expression showed a nonsignificant correlation with both histologic grade and tumor stage.

Conclusion
Our results revealed a limited effect of HER-2/neu on the biologic behavior of gastric carcinoma. However, further study with a larger sample size is recommended to clarify the therapeutic role of trastuzumab (Herceptin) in gastric carcinoma patients expressing HER-2/neu.

Keywords:
gastric carcinoma, HER-2/neu, immunohistochemistry

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Introduction

Gastric cancer is the most common cause of cancer-related death and is considered the fourth most commonly diagnosed neoplasm worldwide [1,2]. Men are twice as likely to be affected with gastric cancer than are women. This may be explained by the protective effect of estrogen against gastric cancer development [3,4].

Many factors may be responsible for gastric cancer. Although *Helicobacter pylori* infection is an important risk factor in most cases of stomach carcinoma, only 2% of patients develop gastric cancer [5]. Smoking is another risk factor increasing the incidence of gastric cancer – from 40% in current smokers to 82% in heavy smokers [6,7]. Dietary factors, although not proved as essential risk factors, such as consumption of red meat, processed meat, smoked food, and salt-rich food, may be correlated with gastric cancer development [8,9].

About 10% of cases occur in families wherein genetic factors constitute the primary cause in 1–3% of cancer cases, such as hereditary diffuse gastric cancer [10].

Adenocarcinomas represent more than 95% of gastric carcinoma cases [11]. Since 1965, the most commonly used classification for gastric cancer is the Laurén classification [12], which groups gastric carcinoma into intestinal and diffuse types [1]. The intestinal type has been shown to be associated with intestinal metaplasia of the gastric mucosa and has been correlated with H. pylori *infection*, which was not established in diffuse type [13,9].

Gastric carcinogenesis is a multistep and multifactorial process. Whereas environmental factors such as

lifestyle and *H. pylori* infection are associated with the intestinal type of gastric cancer, the diffuse type is often related to genetic abnormalities [2].

During gastric carcinogenesis, an accumulation of genetic and molecular abnormalities occurs, including activation of oncogenes, overexpression of growth factors/receptors, inactivation of tumor suppression genes, DNA repair genes and cell adhesion molecules [14], loss of heterogeneity and point mutations of tumor suppressor genes [15]. Understanding of the molecular events and pathways has led to the application of molecular pathology in prevention, early diagnosis, tumor classification, and therapeutic intervention. The application of molecular techniques for testing molecular abnormalities such as human epidermal growth factor receptor 2 (HER-2) expression in cases of stomach carcinoma has had a significant impact on medical practice [2].

HER-2 is a proto-oncogene located on chromosome 17q21 that encodes a transmembrane protein with tyrosine kinase activity, a member of the HER receptor family and involved in signal transduction pathways, leading to cell growth and differentiation [16].

Amplification of the HER-2 gene and overexpression of its product were first discovered in breast cancer and are significantly associated with worse outcomes [17]. In previous studies, HER-2 is also present in several other malignancies, including colorectal cancer, ovarian cancer, prostate cancer, lung cancer, and, particularly, gastric and gastroesophageal cancer [10,13,18].

As one of the first molecular-targeted drugs to be developed, trastuzumab is a monoclonal antibody directed against HER-2. It was first introduced for the treatment of HER-2-positive advanced breast cancer [17]. With the recent introduction of trastuzumab for the treatment of patients with advanced gastric cancer, the clinical demand for HER-2 assessment is rapidly increasing [19]. Patients with gastric cancer highly expressing HER-2/neu showed the greatest benefit from trastuzumab therapy. Therefore, it is now recommended to assess HER-2/neu status in all cases of gastric carcinoma at the time of initial diagnosis [20].

This study aimed to evaluate HER-2/neu expression in gastric carcinomas and to correlate its expression with clinicopathological parameters and other prognostic factors.

Patients and methods

This study included 45 cases of primary gastric carcinoma from patients who underwent radical or partial gastric resection; these patients had been referred to the pathology department at Kasr El-Aini hospitals and a private laboratory between January and December 2007. The Ethical Committee on human research at our Institute approved the protocol for this study.

Each specimen had been fixed in 10% phosphate buffered formalin and routinely processed to be embedded in paraffin blocks. Serial sections of 4 μm thickness were cut from each block and stained as follows:

(1) With hematoxylin and eosin to revise the diagnosis, type, and grade of the tumor, and perform the pathologic staging of the tumor.
   (a) According to Laurén's classification [12], 32 cases were of intestinal type and 13 cases were of the diffuse type.
   (b) All cases were assessed according to the WHO grading system [21]: 25 cases were grade II and 20 cases were grade III.
   (c) All cases were staged according to the TNM staging system of the WHO [21]: three were of tumor stage T2, 40 were of T3, and two were of T4.
(2) Sections were obtained from each case and mounted on super frost Tissue Mounting (TM) slides for immunohistochemical analysis. Sections were deparaffinized and brought to water. Antigen retrieval was performed using citrate buffer (pH 6) in a water bath. Immunohistochemical staining was performed using an automated immunostainer (Dako Cytomation Autostainer S3400; Dako). The primary antibody used was Dako Cytomation rabbit anti-HER-2 polyclonal antibody (Dako Cytomation, a0485) at a dilution of 1 : 50 for 30 min. Binding and detection were performed using the Dako Cytomation En Vision TM peroxides dual link kit (K5007):
   (a) HER-2 positivity was defined as continuous membranous staining in at least 10% of tumor cells.
   (b) A paraffin section of invasive breast carcinoma stained with HER-2/neu was used as a positive control.

Statistical analysis

Data were analyzed using the statistical package SPSS for Windows (IBM, New York, New York, USA). Statistical analysis was performed using Fisher's exact test. *P* values less than 0.05 were considered statistically significant.

Results

In this study, a total of 45 cases of primary gastric carcinoma have been studied. Intestinal type constituted 71% of the cases and diffuse type constituted 29% of the cases.

According to the WHO 2004 grading system, 25 (55.6%) cases were of grade II and 20 (44.4%) cases were of grade III.

According to the TNM staging system, three (6.7%) cases were of tumor stage T2, 40 (88.9%) cases were of stage T3, and two (4.4%) cases were of stage T4.

As regards tumoral N stage, 10 (22.2%) cases were N0, 17 (37.8%) cases were N1, 12 (26.7%) cases were N2, and six (13.3%) cases were N3.

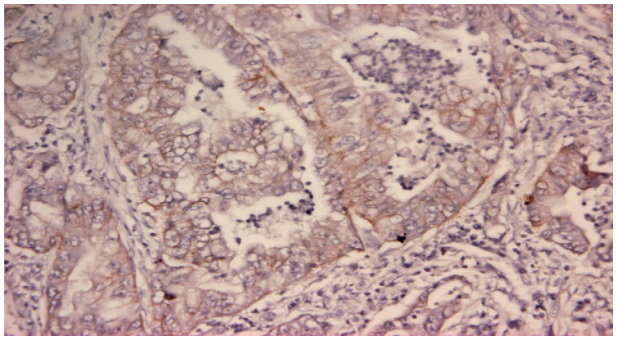
Overexpression of HER-2/neu was detected in five (11.1%) out of 30 gastric carcinoma cases. Stromal and normal epithelial cells adjacent to the tumor tissue were negatively stained (Figs. 1–3).

There was a nonsignificant correlation ($P>0.05$) between HER-2/neu positivity and tumor histological type, wherein 9.4% of intestinal type and 15.4% of diffuse type were HER-2/neu positive (Table 1).

It was found that 12% of grade II and 10% of grade III tumors were HER-2/neu positive, revealing a nonsignificant correlation ($P>0.05$) between HER-2/neu positivity and tumor histological grade (Table 2).

As regards tumor stage, there was a nonsignificant correlation ($P>0.05$) between HER-2/neu positivity and tumor T stage, wherein the five HER-2/neu-positive cases were T3, representing 12.5% of T3 cases (Table 3).

Figure 1



Immunohistochemical staining of human epidermal growth factor receptor 2/neu in grade II intestinal gastric adenocarcinoma with strong membrane staining. Immunohistochemistry, ×400.

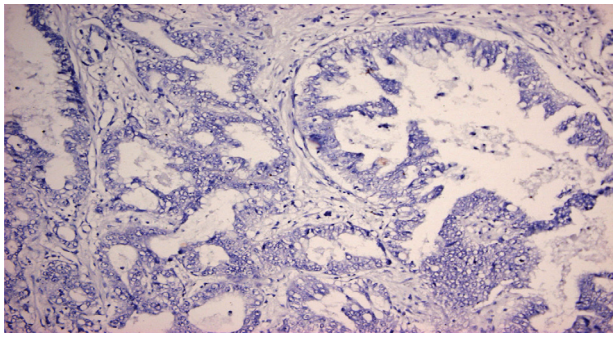
It was also found that tumor N stage was nonsignificantly correlated with HER-2/neu positivity (Table 4).

Discussion

Gastric carcinoma is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related death. It is associated with morbidity and mortality worldwide [22,23]. Although its incidence is persistently declining because of changes in nutrition and better prevention and treatment, gastric cancer with palliative chemotherapy is still associated with a poor prognosis [1]. Recent advances in molecular medicine have not only shed light on the carcinogenesis of gastric cancer but also offered novel approaches regarding prevention, diagnosis, and therapeutic intervention [2].

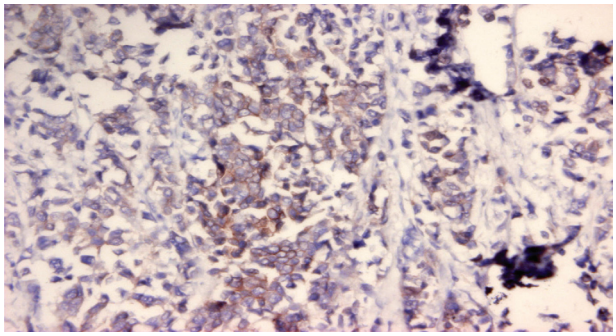
The humanized monoclonal antibody against HER-2, trastuzumab (Herceptin), when combined with chemotherapy (capecitabine or 5-fluorouracil and cisplatin), could effectively prolong overall survival and progression-free survival, and increase the

Figure 3



Infiltrating intestinal gastric adenocarcinoma of the stomach (grade II) showing negative staining for human epidermal growth factor receptor 2/neu. Immunohistochemistry, ×200.

Figure 2



Human epidermal growth factor receptor 2/neu membranous staining in diffuse gastric adenocarcinoma, small cell type. Immunohistochemistry, ×400.

Table 1 Relation between human epidermal growth factor receptor 2/neu positivity and tumor histological type of gastric carcinoma cases

	Intestinal [*n* (%)]	Diffuse (signet ring) [*n* (%)]	Total [*n* (%)]
Positive	3 (9.4)	2 (15.4)	5 (11.1)
Negative	29 (90.6)	11 (84.6)	40 (88.9)
Total	32 (100)	13 (100)	45 (100)

P>0.05, nonsignificant.

Table 2 Relation between human epidermal growth factor receptor 2/neu positivity and grade in gastric carcinoma cases

	Grade II [*n* (%)]	Grade III [*n* (%)]	Total
Positive	3 (12)	2 (10)	5
Negative	22(88)	18 (90)	40
Total	25 (100)	20 (100)	45

P>0.05, nonsignificant.

Table 3 Relation between human epidermal growth factor receptor 2/neu positivity and T stage in gastric carcinoma cases

	T2 [*n* (%)]	T3 [*n* (%)]	T4 [*n* (%)]	Total [*n* (%)]
Positive	0	5 (12.5)	0	5 (11.1)
Negative	3 (100)	35 (87.5)	2 (100)	40 (88.9)
Total	3 (100)	40 (100)	2 (100)	45 (100)

P>0.05, nonsignificant.

Table 4 Relation between human epidermal growth factor receptor 2/neu positivity and N stage in gastric carcinoma cases

	N0 [*n* (%)]	N1 [*n* (%)]	N2 [*n* (%)]	N3 [*n* (%)]	Total
Positive	1 (10)	2 (11.8)	2(16.7)	0	5
Negative	9 (90)	15(88.2)	10(83.3)	6 (100)	40
Total	10 (100)	17 (100)	12 (100)	6 (100)	45

P>0.05, nonsignificant.

response rate in HER-2-positive advanced gastric carcinoma [24].

The response of gastric carcinoma to trastuzumab is predicted well from the levels of HER-2 protein. On the other hand, the tumors with positive HER-2 amplification but with low or negative HER-2 expression do not respond well to trastuzumab. Therefore, immunohistochemistry is recommended to be used as the initial testing methodology, and fluorescence in-situ hybridization or silver *in-situ* hybridization is used to retest immunohistochemistry [25].

The most commonly used classification for gastric cancer was the Laurén classification, which classifies gastric carcinoma into intestinal and diffuse types [1]. In another study, there was an unclassified group known as the atypical group [26]. In our study, 21 out of 30 (70%) cases were of intestinal type, whereas nine (30%) cases were of the diffuse type. This is in agreement with the findings of Ghoshetal [27] and García *et al* [28]. In contrast, a previous study showed that the number of diffuse-type gastric carcinoma cases exceeded that of intestinal type [28,29]. Pinto-de-Sousa *et al.* [30] had classified their cases into intestinal type (51%), diffuse type (29.9%), and unclassified cases (19.1%).

According to the WHO 2004 grading system, 17 (56.7%) cases were of grade II and 13 (43.3%) were of grade III. According to the TNM staging system, two (6.7%) cases were of tumor stage T2, 27 (90%) were of stage T3, and one (3.3%) case was of stage T4.

HER-2/neu is a proto-oncogene located at chromosome 17q21. It is a growth regulatory factor and a cell motility factor that interferes with the signaling cascades involved in cell differentiation, proliferation, and survival [24]. HER-2/neu was proved to be an important biomarker in many cancers, including gastric and gastroesophageal junction tumors [20].

This study aimed to evaluate HER-2/neu expression in gastric carcinomas and correlate its expression with clinicopathological parameters and other prognostic factors.

HER-2/neu was considered positive when expressed as membranous staining in at least 10% of malignant cells. In our study, HER-2/neu immunoreactivity was detected in three out of 30 (10%) cases. Previous studies showed HRT-2/neu overexpression in 7–34% of gastric carcinoma cases [20,25,28–31].

Using various techniques, HER-2/neu expression has been shown to have wide variation in different tumors. It was recommended by expert pathologists that if HER-2/neu expression showed less than 10% strongly stained tumor cells, these cases must be subjected to in-situ hybridization to exclude false-negative results [25,33].

As HER-2/neu is overexpressed in invasive ductal breast carcinoma more than in lobular carcinoma, our study revealed that HER-2/neu expression in intestinal type is nonsignificantly higher than that in diffuse type. This is in agreement with many previous studies [19,29,30]. Other studies found a significant HER-2/neu overexpression in intestinal type [27,33–37]. Although intestinal type showed overexpression for HER-2/neu,

not all intestinal-type gastric carcinomas showed this overexpression. HER-2/neu overexpression cannot be the only factor involved; other external factors may also be involved [28]. In the present study, 11.8% of grade II gastric carcinomas and 7.7% of grade III tumors showed HER-2/neu overexpression, revealing a nonsignificant correlation with histologic grade. Previous studies revealed significant correlation between HER-2/neu overexpression and well-differentiated tumors [28,35], whereas other studies showed positive relation with poorly differentiated ones [27,35].

Several clinical and pathological parameters such as age at onset, tumor location, gastric wall invasion, and distant metastases may predict the prognosis of gastric cancer [27,37]. Also several genetic alterations, including HER-2/neu overexpression, have been reported in gastric carcinoma [36,37].

As regards the prognosis of gastric carcinoma, our study revealed a nonsignificant correlation between HER-2/neu and tumor stage, where the three HER-2/neu-positive cases were T3, representing 11% of T3 cases. This was in agreement with other previous studies [30,35]. Other reports showed HER-2/neu overexpression in advanced cases; however, there was a nonsignificant correlation between positivity and different poor prognostic factors [27]. A significant correlation and close association between HER-2 expression and aggressiveness of the tumor was revealed in previous studies [38,39].Therefore, HER-2/neu expression in gastric carcinomas is related to their aggressive clinical behavior and poor survival rate [35,40].

In addition to the determination of the prognostic role of HER-2/neu expression in gastric carcinoma, fluorescence in-situ hybridization (FISH) or Chromogenic in situ hybridization (CISH) methods could be utilized to detect gene amplification to evaluate patient survival [40].

In conclusion, our results revealed a limited effect of HER-2/neu on the biologic behavior of gastric carcinoma. However, further study with a larger sample size is recommended to clarify the therapeutic role of trastuzumab (Herceptin) in gastric carcinoma patients expressing HER-2/neu.

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Conflicts of interest
There are no conflicts of interest.

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