Human epidermal growth factor 2 status in gastric adenocarcinoma: a retrospective study to assess the susceptibility for trastuzumab treatment

Shaimaa K. Dawa and Eman M.S. Zedan

Objective **We are aiming to study the expression of human epidermal growth factor 2 (HER2) in gastric carcinoma (GC) to estimate the susceptibility for target therapy by trastuzumab hoping to improve the outcome especially in late stage cases.**

Materials and methods **This is a retrospective study that included 50 cases diagnosed as GC in Benha University hospital. Our study included an examination of the endoscopic biopsies of 40 cases and the resection specimens of 10 cases. We assessed HER2 status by application of immunohistochemistry to all the cases. When cases were equivocal on immunohistochemistry, we applied fluorescence in-situ hybridization. The collected data were statistically analyzed.**

Results **HER2 was positive in 17 (34%) cases. It was statistically significant ($P < 0.05$) in the intestinal type of gastric cancer than in the diffuse type (94.1 and 5.9%, respectively). We found the HER2 positivity more in small biopsies than in the resected specimens, but it was statistically nonsignificant. Our data showed no difference in HER2 positivity in relation to the tumor site, differentiation, and tumor stage.**

Conclusion **HER2 is overexpressed in the intestinal subtype of GC. So, this subtype of GC can be treated by target immunotherapy 'trastuzumab'. *Egypt J Pathol* 38:126–130 © 2018 Egyptian Journal of Pathology.**

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Introduction

Worldwide, gastric carcinoma (GC) occupies the fourth position between the most commonly diagnosed cancer. It comes after lung, breast, and colorectal cancers, and it represents 7.8% of the total malignancies. Mortality related to malignant gastric neoplasm is about one million people per year, representing the second most common cause of cancer-related death (Torre *et al.*, 2016). In Egypt, according to statistics of the Egyptian National Cancer Institute, GC represents 1.5% of the total cancer incidence in males, whereas 1.8% in females (Ibrahim *et al.*, 2014).

Pathogenesis of GC is multistep and multifactorial. Although the intestinal type of GC is often linked to environmental factors as *Helicobacter pylori*, diet, and lifestyle. However, the diffuse type is usually related to genetic aberrations (Hohenberger and Gretschel, 2003). Some genetic studies on stomach carcinoma discovered amplifications of the human epidermal growth factor 2 (HER2) gene (Sekaran *et al.*, 2012). Early stage disease standard treatment depends mainly on radical surgery (Hashem *et al.*, 2016). In case of advanced GC and metastatic GC, trials were implemented to use the targeted therapy (trastuzumab for gastric cancer) in combination with the conventional chemotherapeutic agents and found to improve patients' survivals in many countries (Bang *et al.*, 2010). Since this time, many literatures all over the world were done to evaluate HER2 status in GC. In Egypt, only a few published studies are available in this field. The current study was performed to investigate HER2 expression in gastric adenocarcinoma and to correlate between HER2 positivity and several clinicopathological variables.

Materials and methods

This is a retrospective study done at theDepartment of Pathology, Faculty of Medicine, Benha University Hospital from January 2016 to January 2018. The study included 50 gastric adenocarcinoma patients diagnosed between 2010 and 2016. The study consists of 10 cases of gastrectomy resection specimens and 40 endoscopic biopsies. No medication had been given to the patients before diagnosis. Approval of the study protocol taken by the local ethical committee.

Tumor classification and grading

Hemaoxylin and eosin stained slides were revised to confirm the previous histopathological diagnosis. Gastric adenocarcinoma classification and grading were performed according to Lauren's classification. The GC was classified into intestinal type and diffuse type. The intestinal subtype were graded as grade I, II, or III according to the percentage of glandular pattern (95%, 51–94%, or < 50% respectively). In contrast, the diffuse subtype was considered grade III (Lauren, 1965). pTNM classification was done according to the American Joint Committee on Cancer (AJCC), 7th edition (Washington, 2010).

Immunohistochemical method

The collected paraffin blocks were sectioned into four micron thickness and were submitted for immunohistochemistry (IHC) stain. The sections were deparaffinized, and then carried to the water. For antigen retrieval sections were incubated in citrate buffer (6pH) in the microwave at 100°C for 30 min. The process such as blocking of nonspecific antigen, then incubation of

primary antibody against HER2 protein (A0485; Dako, Carpinteria, California, USA) for 1 h, followed by secondary antibody for 30 min was done. For visualization of the reaction, diaminobenzidine (ab143166) was added. The sections were counterstained with hematoxylin (ab143166) and then cover slipped. We added positive as well as negative controls with each IHC run.

Immunostain scoring

Criteria of Hofmann *et al.* (2008), which had been modified by Davidson and Pai (2013) was used as follows:

(1) Positive HER2 (IHC + 3):
   (a) For resection specimen: moderate/strong complete OR basolateral/lateral membranous positivity in greater than 10% of malignant cells. Clear membranous staining at low magnification (at × 4 magnification).
   (b) For small biopsy specimens, any single cluster of tumor cells (≥5 cells) demonstrating IHC 3 + staining characteristics.
(2) Negative HER2:
   (a) IHC 0/negative: no expression (at × 40 magnification) or membranous expression in less than 10% of cells.
   (b) 1 + /negative: faint/hardly detectable partial membranous reactivity in greater than 10% of tumor cells (at × 40 magnification).
(3) Equivocal HER2 (IHC + 2):
   (a) Weak/mild complete OR basolateral/lateral membranous reactivity in greater than 10% of the tumor cells (at × 10magnification).
   (b) Specimens with IHC 2 + were subsequently evaluated by fluorescence in-situ hybridization (FISH) assay to judge HER2 status, then correlated with the clinicopathological variables as age, gender, tumor site, type, and grade, and pTNM classification was undertaken.

Statistical analysis

The collected data and our findings were statistically evaluated using a Microsoft statistical program (SPSS, Version 16; SPSS Inc., Chicago, Illinois, USA). χ^2-Test/Person's correlation was used to investigate the relation of HER2 expression to demographic and pathological variables.

Results

This study included 50 of GC with male to female ratio (2.8 : 1). Patients' mean age was 56 years, with a range from 35 to 77 years. The main presenting symptoms were vomiting (40%), dyspepsia (38%), bleeding (35%), and loss of weight (20%). Tumors were located in the antrum (40%), pylorus (30%), fundus (8%), and involved the whole stomach (linitisplastica) in 22% (Table 1).

Forty (80%) cases were of endoscopic biopsies and 10 (20%) cases were of surgical resection specimens. According to Lauren's classification for tumor typing, 39 cases were intestinal subtype and 11 were diffuse subtype adenocarcinoma. Among the 40 endoscopic specimens, 31 specimens showed intestinal type and nine specimens diffuse type. Out of the 10 cases of surgical resection, eight were intestinal type and two specimens were diffuse type. Most of the intestinal type of gastric adenocarcinoma presented with grade II tumors (27/39 = 69.2%). Among the 10 resection specimens, seven (70%) patients revealed stage III disease. So, most of GC patients who went through surgery were in stage III. Association with, intestinal metaplasia was reported in both endoscopic and surgical specimens. It was seen in 22 out of 50 (44%) patients. *H. pylori* infection associated gastritis was detected in seven (70%) of the surgical specimens.

Table 1 Human epidermal growth factor 2 overexpression and clinicopathological variables in gastric carcinoma patients

Clinicopathological variables	Number	Positive cases [*n* (%)]	*P*-value
Sex			
Male	37	13 (35.1)	0.4
Female	13	4 (23.6)	
Type of specimen			
Small biopsies	40	16 (40)	0.08
Resection	10	1 (10)	
Histological type			
Intestinal	39	16 (41)	0.05 (significant)
Diffuse	11	1 (9)	
Differentiation			
Mod-differentiation	37	13 (35.1)	0.4
Poorly-differentiation	13	4 (23.6)	
Stage of cancer			
Stage II	3	0	0.45
Stage III	7	1 (14.3)	

$P \leq 0.05$, considered as positive.

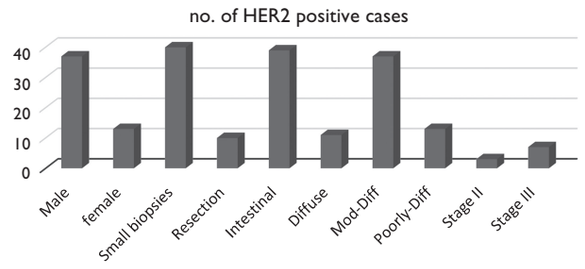
The IHC was performed to evaluate HER2 expression. Among the 50 cases, 15 (30%) patients revealed HER2 score 3 +, and 33 (66%) scored 0 or 1 + (negative). Two (4%) cases had a score of 2 + a (equivocal); and FISH was positive in both. Then the overall positive cases were 17, in which 16 cases of them (94.1%) showed intestinal type and one (5.9%) case displayed diffuse type adenocarcinoma. We found a statistically significant '*P*' value ($P = 0.05$) when calculating the difference in the positivity rate for HER2 between these two histological groups of adenocarcinoma. Out of these 16 HER2 positive cases of intestinal adenocarcinoma, 13 were moderately differentiated (grade II) and three were of high grade (grade III). No grade I cases were found in our study. When we compared HER2 positivity in relation to the type of specimen, we noticed that HER2 overexpression in small biopsies was more (16/40 = 40%) than in the resected specimen (1/10 = 10%), but the '*P*' value was not significant ($P = 0.08$). HER2 positivity rates and its relation to various clinicopathological factors are summarized in Table 1 and Graph 1.

HER2 immunostaining was heterogeneous in all positive cases. There was no difference between the HER2 overexpression (positive) cases and low expression (negative) cases in relation to patient age, gender, as well as tumor site, tumor differentiation, and tumor stage (Fig. 1).

Discussion

GC is one of the most common cancers all over the world. Early stage gastric cancer is treated mainly by surgical resection. Most of GC patients are seeking

Graph 1



Human epidermal growth factor 2 overexpression and clinicopathological variables in gastric carcinoma patients:

medical advice when it is late to be resected and the systemic chemotherapy is the main therapeutic modality for them (Hohenberger and Gretschel, 2003). The survival rate of patients with late stage GC treated with preoperative chemotherapy or adjuvant chemoradiation is still poor (Cunningham *et al.*, 2006).

The present therapeutic modalities for GC patients seems to get a plateau phase of effectiveness and new treatment options, such as targeting agents are required to be investigated. Prognosis of GC depends on many factors as patient's age, tumor stage, specific tumor location, size, and histological type, however, the most crucial of them is tumor stage followed by histological type. Even so, patients with the same stage and histological type exhibit different prognosis, hence new parameters should be clarified to enable assessment of the biological behavior of GC (Aditi *et al.*, 2016).

Pathogenesis of GC is a multistep procedure with variable genetic alterations, including oncogenes stimulation and/or the tumor suppressor genes deactivation (Gravalos and Jimeno, 2008). Previous studies defined many genetic changes related to GC that exhibit HER2 overexpression at the molecular and protein levels, and these tumors may have unique clinicopathological characteristics that may allow optimal therapeutic approaches for them (Aditi *et al.*, 2016).

HER2/NEU gene regularizes HER2 protein synthesis. HER2 protein is one of the epidermal growth factor receptor family. It is a growth factor receptor having intrinsic protein tyrosine kinase action and its increased activity is a supposed mechanism in carcinogenesis. Stimulation of epidermal growth factor receptor activates several signaling pathways that encourages neoplastic cell proliferation, differentiation, migration, adhesion, angiogenesis, and suppress apoptosis. Several studies showed amplifications of the HER2 gene in different carcinomas as of breast, stomach, colon,and they found a poor prognosis in such cases (Sekaran *et al.*, 2012).

Hofmann *et al.* (2008) advised a HER2 immunostaining scoring system in GC for optimal positivity detection and a high level of concordance (93.5%) between IHC and FISH testing.

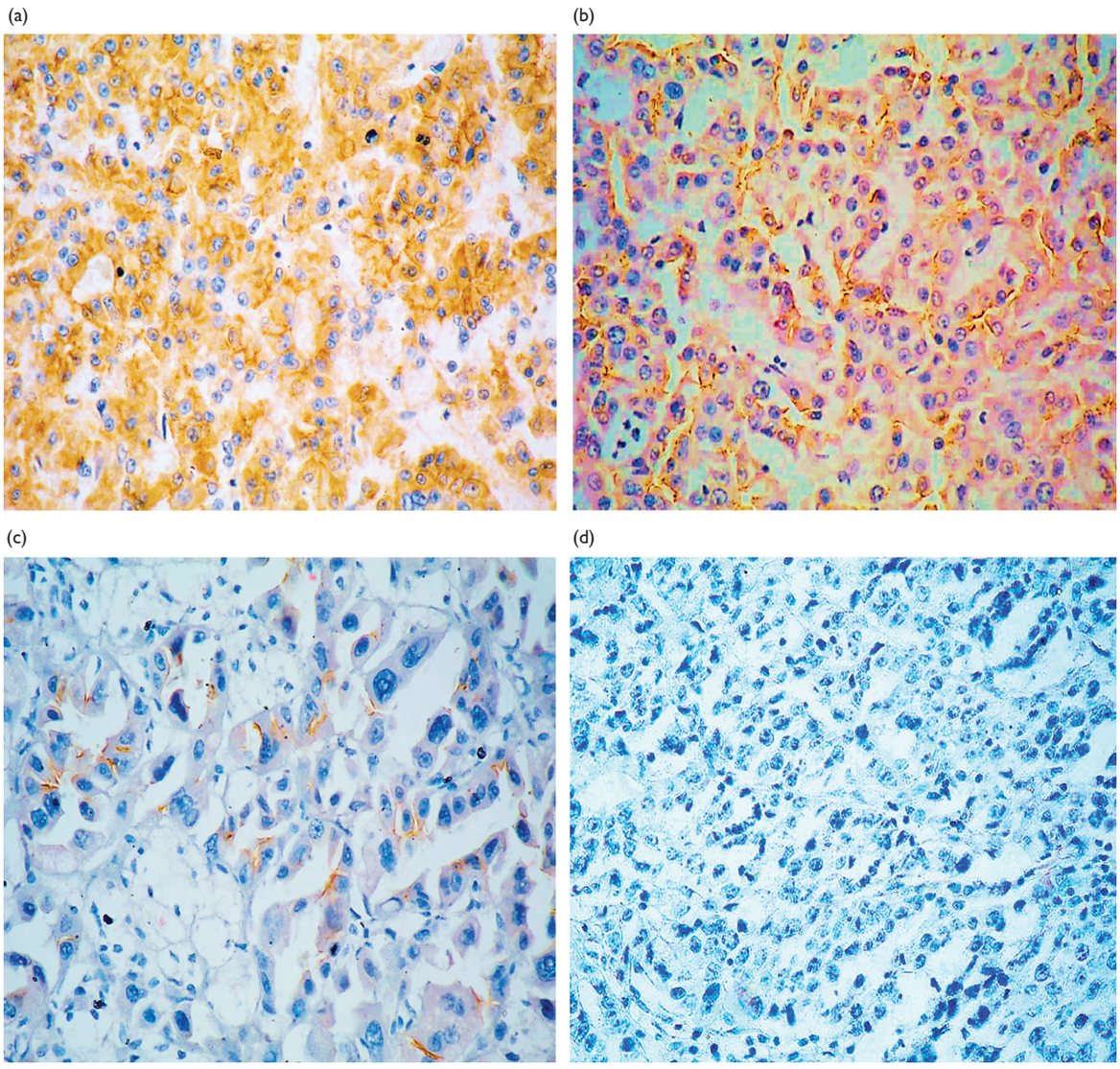
In 2010, trastuzumab for gastric cancer study was fashioned to evaluate the clinical effectiveness and safety of the anti-HER2 agent trastuzumab as an addition to chemotherapy for first-line treatment of advanced or metastatic GC cases overexpressing HER2.These studies revealed the importance of HER2 overexpression in the prognosis and treatments of GC. It has been authorized as therapeutic agent in HER2 positive metastatic intestinal type GC. Therefore, HER2 condition should nowadays be regularly checked in the investigation of patients who had a late stage GC (Bang *et al.*, 2010).

The current study included clinical characteristics, histology, and HER2 status in GC cases diagnosed in Benha and Asute university hospitals over a period of 24 months. Adenocarcinoma of the stomach has been found to demonstrate a broad degree of variations in HER2 overexpression from (10.7 and $\geq$44.2%) with respect to different ethnic groups. A study on Germans showed 19% positive cases in total of 166 cases (Marx, *et al.*, 2009). A study on Chinese patients revealed 18.8% positive cases in a total of 218 cases (Xie *et al.*, 2009). Another study that included Germans, Chinese, and Mexicans patients found 10.7% positive HER2 in 178 cases (Hofmann *et al.*, 2008). A study on Iranian patients detected 26% positive HER2 in 100 cases (Raziee *et al.*, 2007), whereas on Australians it was 17.4% ($n=178$) (Lee *et al.*, 2011). The latter was near to the study result performed on Japanese patients as it was 17% in 207 cases (Yoshida *et al.*, 2014). In India, the positive cases ranged from 44.2% ($n=52$) (Sekaran *et al.*, 2012), 35.9% ($n=78$) (Lakshmi *et al.*, 2014) to 27.6% ($n=58$) (Aditi *et al.*, 2016). In Nigerians, it was 11% ($n=36$) (Ogun *et al.*, 2014). Hashem *et al.* (2016) carried out a study on Egyptian patients. They found that HER2 was positive in 4 out of 39 patients (10.3%). Our results showed HER2 overexpression in 17 out of 50 GC cases (34%). This variation between studies may be due to multiple factors as difference in procedure/ method, applied standard, reporting regimen differences, tumor heterogeneity, or variations of HER2 positivity in the populations studied.

Yildiz-Aktas *et al.* (2012) found that amplification of HER2 gene was common in invasive ductal carcinoma of the breast, but rare in lobular carcinoma. Also,in our study, it is significantly overexpressed in the intestinal type of GC (16/17 = 94.1%) in comparison to the diffuse type (1/17 = 5.9%; $P=0.05$). Our finding agrees with many other studies in which HER2 positivity was more common in the intestinal type than the diffuse type (83.3%) according to Hofmann *et al.* (2008), (74%) by Raziee *et al.* (2007), (67.9%) in Kim *et al.* (2007), 92% in Marx, *et al.* (2009), (95%) detected in Tafe *et al.* (2011), (84.6%) reported by Lakshmi *et al.* (2014), and (93.8%) by Aditi *et al.* (2016). All these findings may impose the hypothesis that different histologic tumor types may develop after different genetic alterations. For instance, E-cadherin status is reduced or abnormal in the diffuse type of GC. However, these results are in contrast to those of Sekaran *et al.* (2012) who found equal HER2 positivity in both types.

Out of the 40 endoscopic biopsies collected in the current study, 16 (40%) cases showed HER2 positivity, whereas only in one out of 10 cases the resected

Fig. 1



Different HER2 expression in gastric carcinoma. (a) HER2 (score + 3) in moderately differentiated intestinal type gastric adenocarcinoma (ABCx200). (b) HER2 (score + 2) in moderately differentiated gastric carcinoma (ABC × 200). (c) HER2 (score + 1) in poorly differentiated gastric adenocarcinoma, intestinal type (ABC × 400). (d) HER2 (score + 0) in gastric carcinoma diffuse type (ABC × 200). HER2, human epidermal growth factor 2.

specimens (10%) showed HER2 positivity. Therefore, a much higher rate of positivity in endoscopic specimens in comparison with the resected specimens were observed. However, this higher rate of positivity was not statistically significant between both the groups. This finding has also been reported by Lee *et al.* (2011), Jeung *et al.* (2012), and Aditi *et al.* (2016). Davidson and Pai (2013) had supposed that as endoscopic biopsy was quickly fixed, it had shorter cold ischemic time that leads to more antigen preservation and hence a higher rate of positivity. Cold ischemic time is defined as the time lag between removal of tissue from the living body and the start of formalin penetration in the tissue. This explained that tissue ischemia induced by surgical interruption of blood supply leads to advancing loss of labile macromolecules activity due to acidosis and degradation by enzymes (Yaziji *et al.*, 2008). Also, Yildiz-Aktas *et al.* (2012) supported this theory and suggested that it is of top-quality to make cold ischemic time as less as achievable, and they recommended less than one hour as a wise guideline to follow studying the consequence of cold ischemic time on HER2 expression in mammary carcinoma resection specimens. Yet, pursuing this guideline will necessitate modifications in practice, and in many organizations teamwork and cooperation will be required. The surgeons should refer resection specimens quickly to pathology laboratories, pathology laboratories must assign a pathology assistant, for promptly grossing large specimens to section the tumor directly and then keep it in buffered formalin for overnight fixation. Thus, in this study, it is better to perform IHC on small biopsies as it is more sensitive to HER2 when treatment with trastuzumab is available. In contrast, Yoshida *et al.* (2014) who used IHC and FISH to study the HER2

status, reported no significant differences between the resection group and biopsy group. Concerning the relation of HER2 expression to tumor differentiation, Kim *et al.* (2007) study found a higher rate of HER2 overexpression in moderately differentiated cases, (51.8%). Similarly, Marx *et al.* (2009) reported that 60% of HER2 positive cases were moderately differentiated. Also our study showed that 94.1% of positive cases were moderately differentiated. Low grade carcinoma in most of the studies showed a very low rate of HER2 expression (Tateishi *et al.*, 1992; Ross and McKenna, 2001).

Conclusion

Our study recommends further investigation on HER2 expression in GC samples, especially the intestinal type to give a chance for GC patients, especially those in late stage to benefit from the target therapy using trastuzumab.

Conflicts of interest

There are no conflicts of interest.

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