

# A study of human epidermal growth factor receptor 2 overexpression by immunohistochemistry in patients with gastric adenocarcinoma

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**Abstract.** Gastric cancer is one of the leading causes of cancer-related deaths across the world and in the Middle East. Human epidermal growth factor receptor 2 (HER2) overexpression has been observed in gastric cancers. Trastuzumab, a recombinant monoclonal antibody targeting HER2 protein, is being used for treatment of metastatic gastric cancer. To study the frequency and association of HER2 overexpression with age, gender, histopathological subtype and grade of differentiation in patients with gastric adenocarcinoma from Basra, Iraq. This cross-sectional single-center study collected demographic (age, gender), histopathological (histological subtype, grade of differentiation) and immunohistochemical (HER2 overexpression status) data from 100 consenting adult patients (male: 56) with histopathologically confirmed gastric adenocarcinoma from samples obtained through endoscopy or surgery. HER2 overexpression (ToGA score 3+) was observed in 6/100 (6%) of patients, with another 6 showing 'equivocal' HER2 expression (2+). Out of 20 patients with moderately differentiated gastric cancer, 4 (20%) showed HER2 overexpression (P=0.008). Other factors considered (age, gender, histological subtype) did not show statistically significant correlation with HER2 overexpression. More females showed HER2 overexpression than males (4 vs. 2), and more patients with intestinal type gastric cancer showed HER2 overexpression than diffuse gastric cancer (5 vs. 1), but the difference was not statistically significant in both variables. HER2 overexpression was 6% in this population; statistically significant correlation was found with histological grade. Statistically non-significant correlations were observed between HER2 overexpression and gender, age, and histological subtype.

## Introduction

Gastric cancer is the fourth most common cancer worldwide, and ranks second among the most common causes of cancer-related deaths (1). Even in the Middle-East, gastric cancer is highly prevalent and is an important cause of mortality (2,3). A large proportion of patients with gastric cancer present at later stage, when the malignancy would have become metastatic and inoperable, and hence is associated with 5-year survival rates as low as 5-20%, and a low median overall survival of less than 1 year (4,5). Since surgery is the mainstay of treatment of gastric cancer, conventional chemotherapeutic options are also not beneficial for such patients with inoperable cancers (6,7).

In an effort to identify novel targets for better treatment options of patients with advanced gastric cancer, the role of human epidermal growth factor receptor 2 (HER2) came under scrutiny. The HER2 is a proto-oncogene encoded by *ERBB2* gene located on chromosome 17. The major role of HER2 is to suppress apoptosis and promote cell proliferation in the tissues where it is expressed, most notably breast, gastrointestinal tract, kidney, and heart. The net outcome of this action is to promote tumorigenesis by facilitating uncontrolled cell growth (8). Trastuzumab, which is a humanized monoclonal antibody targeting HER2 receptor has significantly improved the treatment outcomes of HER2-positive breast cancer (8). Subsequently, it was recognized that HER2 status has significance in severe forms of other cancers, notably gastric cancer. This led to the publication of landmark studies such as the Trastuzumab for Gastric Cancer (ToGA) trial in 2010 (9), which subsequently led to the approval of trastuzumab for the treatment of advanced gastric cancer with confirmed HER2 positivity status by means of immunohistochemistry (IHC) and/or fluorescent in-situ hybridization (FISH) technique (10).

There is a shortage in studies describing the frequency and clinico-pathological features with HER2 overexpression in Iraqi patients with gastric cancer. So, the study was designed to detect how frequent the HER2 overexpression in Iraqi patients with gastric cancer and its clinico-pathological correlation.

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## Materials and methods

This was a single-centre, cross-sectional, observational study conducted at Basra Oncology and Haematology Centre, Iraq from January 2014 to August 2015. After institutional ethics committee approved the study protocol, consenting adult patients who were diagnosed with gastric adenocarcinoma (either by endoscopic biopsy or histopathological examination of surgically resected specimen) were included in the study. Patients taking neo-adjuvant chemotherapy or radiotherapy and those with histopathological subtypes other than adenocarcinoma were excluded.

Data collected from the patients included age at the time of diagnosis, gender, histopathological subtype (intestinal or diffuse, by Lauren's classification), grade of differentiation (WHO criteria), and HER2 expression status through IHC. The histopathology and IHC reports were examined by two expert histopathologists in a double-blind fashion, and any differences between the pathologists' opinions were resolved by referring the slides to the central histopathological laboratory of the Institution.

**Histopathology and IHC:** all tumor samples were fixed in 10% neutral buffered formalin for 18-24 h and embedded in paraffin, and routinely diagnosed in the Department of Pathology, Basra Oncology and Haematology Centre, Iraq. Immunohistochemical staining was performed according to the manufacturer's guidelines on 4-5  $\mu$ m thick formalin-fixed unstained, air-dried, paraffin-embedded tissue sections, using the commercially available rabbit anti-human HER2/neu protein and reagent kit (DAKO Hercep Test™) for DAKO auto Stainer (Code No. K5207 (IVD)), which is a semi-quantitative immunohistochemical assay for HER2 protein. HER2 IHC was scored following the ToGA scoring scheme described by Hofmann *et al* (11) and validated by Ruschoff *et al* (12) as follows: 0, No membranous reactivity in <10% of tumor cells; 1+, faint membranous reactivity in  $\geq$ 10% of tumor cells (cells are reactive only in part of their membrane); 2+, weak to moderate complete, basolateral or lateral membranous reactivity in  $\geq$ 10% of tumor cells; and 3+, strong complete, basolateral or lateral membranous reactivity in  $\geq$ 10% of tumor cells. Samples with a score of 0 and 1+ were classified as 'negative' for HER2 overexpression, and those with a score of 3+ were classified as 'positive' for HER2 overexpression. Samples with a score of 2+ were classified as 'equivocal' for HER2 overexpression.

All data were entered electronically. Pearson Chi-square test was performed to analyze associations between HER2 status and the different variables, and P-values of less than 0.05 were considered significant. SPSS statistical software program version 20 was used for statistical analysis. (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

## Results

A total of 100 patients were included in the study for analysis, of which 56 were male. The mean age was 53.9 years (range, 17 to 85 years), and around one-third were aged above 60 years. An equal proportion of patients had diffuse gastric type and intestinal type of gastric cancer. The tumor was

poorly differentiated in 69 patients, moderately differentiated in 20 patients, and well-differentiated in 11 patients. The details of HER2 overexpression status categorised by gender, age, histological subtype and grade is presented in Table I.

Our study showed that 6 out of 100 patients with histologically confirmed gastric adenocarcinoma had 'positive' HER2 overexpression by IHC (ToGA score 3+), making the frequency of HER2 overexpression at 6%. Out of the remaining patients, the HER2 overexpression status was 'negative' (0 and 1+ scores) for 88 patients, and 'equivocal' (2+ score) for 6 patients. The HER2 overexpression status had statistically significant association with histological grade and differentiation of the cancer. Even though HER2 positivity was observed more frequently with female patients (when compared to male patients), patients aged <30 years (when compared to other age groups) and with intestinal type gastric cancer (when compared to diffuse gastric cancer), these associations were not statistically significant.

## Discussion and conclusions

The first description of an association between HER2 overexpression and gastric cancer was published in 1986. Ever since, published literature from across the world has reported HER2 overexpression status in gastric cancer patients to range widely between 4.4 and 53.4%, with a mean of 17.9% (13). In our study, HER2 overexpression was observed in 6% of patients, with another 6% showing equivocal results. In a study reported previously from Iraq involving 30 patients, 9 (30%) showed HER2 positivity, but this figure was inclusive of both equivocal and positive scores (14). The numbers from our study thus fall within the wide range as reported in previous studies, but towards the lower end of the spectrum.

Most of the tumors in our study (69%) were poorly differentiated. This observation is consistent with the natural history of the disease, which most often presents in advanced stages. A statistically significant correlation of HER2 overexpression was observed with moderately differentiated cancers. Such a correlation has been described previously as well (15,16). However, there are studies which have reported no such association between grade of differentiation and HER2 overexpression (17,18). The reasons contributing to this conflicting nature of data may include differences in sample sizes, geographical and genetic variations, and different scoring system used. Perhaps the future studies with larger sample sizes and meta-analyses of many similar studies may provide a strong data towards the existence of such a correlation.

Studies published in the past from different parts of the world have reported a significant association of HER2 overexpression with intestinal type of gastric cancer (15,16,19). We observed a trend of HER2 overexpression being associated more frequently with intestinal type cancers when compared to diffuse gastric type cancer, though this association was not statistically significant. A similar trend which was not statistically significant, has also been observed in a study from India (18).

We observed that only 2 patients aged above 60 years overexpressed HER2, even though this age group constituted 33/100 cases in our study. There was also no statistically significant relation between HER2 overexpression and age

Table I. Human epidermal growth factor receptor 2 expression status categorized by different parameters.

Pathologic feature	Total (N)	HER2 overexpression assessment			P-value <sup>a</sup>
		Positive (N, %)	Equivocal (N, %)	Negative (N, %)	
Overall	100	6 (6)	6 (6)	88 (6)	
Gender					
Male	56	2 (3.6)	2 (3.6)	52 (92.9)	0.261
Female	44	4 (9.1)	4 (9.1)	36 (81.8)	
Age group					
<30 years	7	1 (14.3)	1 (14.3)	5 (71.4)	0.735
30-40 years	9	1 (11.1)	0	8 (88.9)	
40-50 years	23	1 (4.3)	0	22 (95.7)	
50-60 years	28	1 (3.6)	2 (7.1)	25 (89.3)	
>60 years	33	2 (6.1)	3 (9.1)	28 (84.8)	
Gastric cancer subtype					
Diffuse gastric cancer	50	1 (2.0)	2 (4.0)	47 (94.0)	0.161
Intestinal type gastric cancer	50	5 (10.0)	4 (8.0)	41 (82.0)	
Histological grade and differentiation					
Well differentiated (Gr. I)	11	1 (9.1)	1 (9.1)	9 (81.8)	0.008
Moderately differentiated (Gr. II)	20	4 (20.0)	3 (15.0)	13 (65.0)	
Poorly differentiated (Gr. III)	69	1 (1.4)	2 (2.9)	66 (95.7)	

<sup>a</sup>P-value by pearson chi-square test.

group. This observation is consistent with most of the studies published recently (15,16,18). This suggests that age plays a minor role, if at all, as an independent factor for initiating Trastuzumab therapy in gastric cancer patients.

An interesting finding from our study is that HER2 overexpression was seen more often in females, in a statistically non-significant manner. In contrast, most of the previously published studies report that more males than females have HER2 overexpression (15,16,18). Further studies with larger sample sizes are required to explore whether there is a gender variation to HER2 overexpression in this geographical area.

The main limitation of our study was the small sample size. Also, we were not able to include FISH, in addition to IHC, for HER2 overexpression assessment. Finally, since our study was a cross-sectional study, we were unable to collect data to correlate HER2 overexpression with factors such as outcomes (beneficial and adverse) of trastuzumab therapy and prognosis.

To conclude, in patients with gastric adenocarcinoma, HER2 overexpression is documented in 6% of patients, with a further 6% of patients showing equivocal HER2 status. There was a statistically significant correlation of HER2 overexpression with histological grade and differentiation of the tumor, but other factors (such as gender, age, and histological type) did not show such a statistically significant correlation. More female patients than males showed HER2 overexpression. In the backdrop of significant variation of findings of our study when compared to similar studies in the past, further studies with larger sample sizes and more criteria are required to validate the findings of our study.

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## Availability of data and materials

Data and materials are available from the corresponding author upon request.

## Conflict of interest

The authors declare no potential conflict of interest.

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