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Immunohistochemical Expression of Her2/neu in Gastric Carcinomas in Egyptian Patients

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Abstract

Background: Gastric cancer is associated with substantial morbidity and mortality worldwide. The frequency of Her2/neu overexpression in gastric carcinoma varies widely in the literature. Furthermore, studies have yielded inconsistent findings regarding its prognostic relevance. In Egypt, few studies about Her2/neu expression in gastric carcinoma were conducted. So, the aim of this study is to evaluate HER-2/neu expression in gastric carcinomas and to investigate its relation with the clinicopathological characteristics in respectable gastric cancer in Egyptian patients.

Methods: This study were conducted on 76 gastric cancer patients who underwent radical gastrectomy at Gastroentrology Center, Mansoura, Egypt from 2007 till 2013. All clinicopathological data were revised. Four TMA blocks were made by Beecher manual microarrayer from formalin-fixed, paraffin-embedded samples of tumors. Immunohistochemical staining of Her2/neu was performed on 4 µm thick sections that were cut from TMA blocks.

Findings: Forty one cases were positive (53.9%) while 35 cases were negative (46.1%). The only statistical significant relation was found between positive Her2/neu expression and Lauren intestinal type. Her2/neu positive expression was associated with poorly differentiated grade, late pathological stage, more depth of tumor invasion and increasing number of LN metastasis but this association does not reach statistical significance. There was no association between HER2/neu positivity and the other clinical pathologic parameters.

Conclusion: The rate of HER2/neu positivity in resectable gastric cancinomas was high (about 54%). The only statistical significant relation was found between positive Her2/neu expression and Lauren intestinal type.

Keywords: Gastric cancer; Her2/neu; Carcinoma; Tumor

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Introduction

Gastric cancer is associated with substantial morbidity and mortality worldwide. In Egypt, gastric cancer is the 9 th most common cancer representing 1.7% of total malignancy in males [1]. Gastric carcinoma is the most common malignant tumor representing 90 to 95% of gastric cancer cases. Histopathologically, gastric carcinoma can be classified according to many classification systems as Lauren classification [2] and WHO classification [3]. Gastric carcinoma is classified histologically by Lauren, into two principal types: intestinal-type (53% to 60%) and diffuse-type (30%) in addition to mixed or indeterminate types (10%). WHO classification (2010) recognizes four major types of gastric carcinoma, namely papillary, tubular, mucinous and signet ring cell carcinomas.

Human epidermal growth factor receptor-2 (Her2/neu) is a proto-oncogene located on chromosome region 17q21. It encodes transmembrane tyrosine kinase receptor protein that regulates signal transduction in cell proliferation, differentiation and

survival [4]. HER2 gene amplification and protein overexpression in gastric cancer were first reported in 1986 [5]. With the recent introduction of trastuzumab for the treatment of patients with advanced gastric cancer, the clinical demand for Her2/neu assessment is rapidly increasing for selecting patients eligible for this treatment [6]. Her2/neu testing in gastric cancer differs from testing in breast cancer because of inherent differences in tumor biology as incomplete membrane staining and intratumoral heterogeneity of Her2/neu expression that are commonly observed in gastric tumors [7]. In gastric cancer, the frequency of Her2/neu overexpression varies widely in the literature from about 4.4% to 53.4% [8]. Furthermore, studies have yielded inconsistent findings regarding its prognostic relevance; the majority indicated that Her2/neu showing more aggressive biological behavior and higher frequencies of recurrence while few studies have not demonstrated the prognostic significance of Her2/neu [9]. In Egypt, few studies about Her2/neu expression in gastric carcinoma were conducted. So, the aim of this study is to evaluate HER-2/neu expression in gastric carcinomas and to investigate its relation with the clinicopathological characteristics in resectable gastric cancer in Egyptian patients.

Methods

This retrospective study were conducted on 76 gastric cancer patients who underwent radical gastrectomy at Gastroentrology Center, Mansoura, Egypt from 2007 till 2013. All clinicopathological data were revised and included: age, gender, location, size, shape, histological type according to WHO and Lauren' classification, WHO tumour grade, depth of invasion (T), lymphovascular invasion, perineural invasion, number of lymph node (LN) metastases, distant metastasis and TNM staging.

Four tissue microarray (TMA) blocks were made by Beecher manual microarrayer from formalin-fixed, paraffin-embedded samples of tumors. Immunohistochemical staining was performed on 4 µm thick sections that were cut from TMA blocks and then mounted on coated slides. DAKO kit (Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse, Produktionsvej 42, DK-2600, Glostrup, Denemark) was used. The horseradish peroxidase an diaminobenzedene hydrochloride (DAB) are the enzyme and chromogen employed. A distinct brown membranous stain was considered as positive. The percentages of Her2/neu immunopositive tumor cells in area that showed the highest density of these cells was determined in each core.

A cutoff value of <10% immunopositive cells was considered negative, faint incomplete membranous or basolateral staining of more than 10% of tumor cells were considered as negative (Score 1), Weak to moderate membranous or basolateral staining of ≥ 10% immunopositive cells was considered positive (+2). Strong complete membranous or basolateral staining of ≥ 10% immunopositive cells is considered as positive (+3) [10]. Cases with score (0) and (1) were considered negative while cases with score (2) and (3) were considered positive.

Results

Demographic data of the patients

Forty seven cases were male (70%) and 29 of cases were female (30%). The range of age of the studied patients was 17-76 years with mean age 52 ± 12.4 years. The fifth decade was the most common age group (28 cases, 36.8%), followed by the sixth decade (21 cases, 27.6%) and the lowest age incidence was below twenty years (one case, 1.3%).

Table 1 Showed the pathological features of tumours of the studied cases.

Pathological features	NO	%
Tumor location		
Fundus	23	30.2
Body and antrum	48	63.2
Pylorus	5	6.6
Gross features of the tumor		
Fungating mass	31	40.7
Malignant ulcer	24	31.5
Annular growth	21	27.6
Size of the tumor		
1-3 cm	14	18.4
4-7 cm	45	59.2
>7 cm	17	22.4
Histopathological type (WHO classification)		
Tubular adenocarcinoma	47	61.8
Signet ring and poorly cohesive carcinoma	21	27.6
Mucinous carcinoma	5	6.6
Others	3	3.9
Histopathological types (Lauren`s classification)		
Intestinal type	47	61.8
Diffuse or mixed type	29	38.2
WHO grade*		
Well-differentiated	5	10.6
Moderately differentiated	29	61.7
Poorly differentiated	13	27.6
Pathological stage: (TNM)		
I	4	5.3
II	39	51.3
III	26	34.2
IV	7	9.2
Depth of invasion		
T2	44	57.9
T3	28	36.8
T4	4	5.3
LN Metastasis		
No	22	28.9
1-5	29	38.2
6-9	20	26.3
>9	5	6.6
Lymphovascular tumor emboli		
Present	27	35.5
Absent	49	64.4
Perineural invasion		
Present	14	18.4
Absent	62	81.6

"Others included 2 cases of neuroendocrine carcinoma and one case of mucoepidermoid carcinoma. *Grading was done for cases of tubular adenocarcinoma only (47 cases)

Table 2 Relation between Her2/neu expression and different clinicopathological features of the studied gastric carcinomas.

Clinicopathological features	Her2/neu –ve cases	Her2/neu +ve cases	X^2	P value
Sex				
Male	22 (46.8%)	25 (53.2%)	0.0283	0.86
Female	13 (44.8%)	16 (55.2%)		
Age groups				
10-19	1 (100%)	0 (0%)	1.661	0.94
20-29	1 (50%)	1 (50%)		
30-39	2 (40%)	3 (60%)		
40-49	13 (46.4%)	15 (53.6%)		
50-59	10 (47.7%)	11 (52.3%)		
60-69	5 (41.6%)	7 (59.4%)		
70-79	3 (42.8%)	4 (57.2%)		
Tumor location				
Fundus	15 (65.2%)	8 (34.8%)	5.977	0.05
Body and antrum	17 (35.2%)	31 (64.6%)		
Pylorus	3 (60%)	2 (40%)		
Gross features				
Fungating mass	14 (45.2%)	17 (54.8%)		
Malignant ulcer	11 (45.8)	13 (54.2%)	0.3112	0.98
Annular growth	10 (47.6%)	11 (52.4%)		
Size of the tumor				
1-3 cm	6 (42.9%)	8 (57.1%)	0.0713	0.965
4-7 cm	21 (46.7%)	24 (53.3%)		
>7 cm	8 (47.1%)	9 (52.9%)		
Histopathological type (WHO classification)				
Tubular adenocarcinoma	17 (36.2%)	30 (63.8%)		
Signet ring and poorly cohesive carcinoma	12 (57.1%)	9 (42.9%)	5.72	0.126
Mucinous carcinoma	4 (80%)	1 (20%)		
Others	2 (66.7%)	1 (33.3)		
Histopathological type (Lauren`s classification)				
Intestinal type	17 (36.2%)	30 (63.8%)	4.842	0.03*
Diffuse or mixed type	18(62.1%)	11 (37.9%)		
WHO grade*				
Well differentiated	1 (20%)	4 (80%)		
Moderately differentiated	11 (38%)	18 (62%)	0.535	0.827
Poorly differentiated	5 (38.5%)	8 (61.5%)		
Pathological stage (TNM)				
I	2 (50%)	2 (50%)	6.612	0.08
II	20 (51.3%)	19 (48.7%)		
III	12 (46.2%)	14 (46.2%)		
IV	1 (14.2%)	6 (85.7%)		
Depth of invasion				
T2	22 (50%)	22 (50%)	4.052	0.25
T3	13 (46.4%)	15 (53.6%)		
T4	0 (0%)	4 (100%)		
LN Metastasis				
No	11 (50%)	11 (50%)		
1-5	13 (44.8%)	16 (55.2%)	0.2381	0.971

6-9	9 (45%)	11 (55%)		
>9	2 (40%)	3 (60%)		
Lymphovascular emboli				
Present	11 (40.7%)	16 (59.3%)	0.4756	0. 49
Absent	24 (49%)	25 (51%)		
Perineural invasion				
Present Absent	4 (28.6%) 31 (50%)	10 (71.4%) 31 (50%)	2.111	0.14

"Others included 2 cases of neuroendocrine carcinoma and one case of mucoepidermoid carcinoma; *P value <0.05 is significant; *Grading was done for cases of tubular adenocarcinoma only (47 cases)

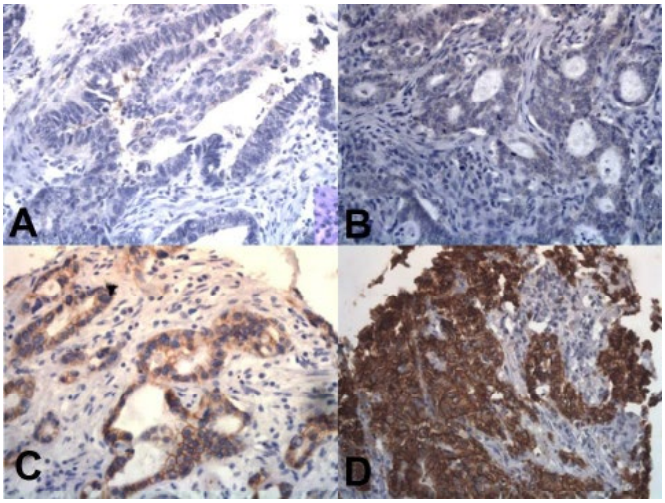

Figure 1 Immunohistochemical staining of Her2/neu in gastric carcinoma (400X). (A) No membranous lateral or basolateral staining of tumour cells (score 0). (B) Incomplete faint membranous, lateral or basolateral staining in <10% of tumour cells (score 1). (C) Moderate membranous, lateral or basolateral staining in >10% of tumour cells (score 2). (D) Complete membranous, lateral or basolateral staining of tumour cells (score 3).

Pathological features of tumors of the studied cases

All the pathological features of the tumors were illustrated in **Table 1**. The body and antrum were the most common tumour location (48 cases, 63.2%). Fungating mass was the most common gross features of the tumours (31 cases, 40.7%).

Her2/neu immunohistochemical results

Forty one cases were positive (53.9%) while 35 cases were negative (46.1%) **(Figure 1).** The only statistical significant relation was found between Her2/neu expression and histopathological type according to Lauren classification being more expressed in intestinal type (P=0.03) **(Table 2)**. We observed higher percentage of HER-2/neu positivity among poorly differentiated grade (61.5% of poorly differentaied tumors were HER-2/neu positive versus 38.5% were HER-2/neu negative), but without statistical significance (p=.827). Similarly, Her2/neu positive expression was associated with late pathological stage (85.7% of stage VI were HER-2/neu positive versus 14.3% were HER-2/neu negative), more depth of invasion of the tumor (53.6% of pT3 were HER-2/neu positive versus 46.4% were HER-2/neu negative), increasing number of LN metastasis (60% of cases that have >9 LN metastasis were HER-2/neu positive versus 40% were HER-2/neu negative) **(Table 2)**. However, this association does not reach statistical significance.

There was no significant difference between Her2/neu positive and negative patients in terms of sex (p=0.86), age of the patients (p=0.94), tumor location (p=0.05), gross features (p=0.98), size of the tumor (p=0.965), histopathological type of the tumor according to WHO classification (p=0.126), lymphovascular emboli (p=0.49), perineural invasion (p=0.14) **(Table 2).**

Discussion

Over expression of Her2/neu molecule in gastric cancer and its correlation with the outcome of the disease has been a subject of interest over the last decade. In this study, we studied Her2 expression in 76 specimens of gastric carcinoma who underwent radical gastrectomy. The rate of Her2/neu positivity in our study is estimated to be about 54% which is more than double the amount of positive tumors reported by Giuffrè et al. who encountered a Her2 overexpression rate of 21.10% in a small cohort of gastric adenocarcinomas [11]. Also, the overall HER2/neu positivity rate was 28.8% and 11.1% in two recent Egyptian study [12,13]. This could be explained by 1-In this study, we considered all cases with score 2+ and 3+ cases as positive as we did not perform Fluorescence in Situ Hybridization due to financial limitations. 2-These results are difficult to be compared with ours due to methodological differences, as we used TMA sections and they used whole tissue sections. 3-Ishaky et al. [13] defied HER-2 positivity as continuous membranous staining in at least 10% of tumor cells but in this study, we considered complete membranous or basolateral staining of ≥ 10% immunopositive cells as positive.

Overexpression of Her2/neu in gastric cancers varies from 8.2% to 62.5% in different reports [14]. This discrepancy of frequency of Her2/neu positivity could be related to different population. Also, the application of different scoring criteria for immunostained slides of gastric adenocarcinoma may emerge to be an important cause in this different range [15]. Also, the discrepancy of these results may be due to methodological differences, especially when TMAs and tumoral protein expression heterogeneity are confronted. In addition, it appears unlikely that all intestinal-type tumors develop as a result of the same external causes, and this

may explain the differing prevalence of HER-2/neu overexpression reported in various studies [16].

The Laurén's intestinal subtype has been proven to be the pathological feature most invariably associated with HER-2/neu positivity in multiple studies including the ToGA trial [17]. Similarly, our study showed significant difference between Her2/neu overexpression in Laurén's intestinal and diffuse gastric carcinomas being more expressed in intestinal type. This may explain the high percentage of Her2/neu positive tumors in this study cohort (about 54%), considering the strong association between intestinal type and Her2/neu positivity and high percentage of Laurén's intestinal subtype in our cases (about 62% of cases). In a recent Egyptian study conducted by Hashem et al. [18], Her-2 was found to be overexpressed in about 10% of cases. Their cohort showed an absolute predominance of the Laurén's diffuse-type which may explain this small percentage of Her2/neu positivity.

Although Her2/neu has emerged as a new therapeutic target in gastric cancer, its role as a prognostic marker in this tumor is still controversial. Indeed, some studies demonstrated that HER-2/neu overexpression is a poor prognostic factor in gastric cancer [19]. In this study, Her2/neu positive status was associated with clinicopathological features of tumor progression, such as higher grade, late stage, more depth of invasion and increasing number of LN metastases but this association does not reach statistical significance. In advanced gastric cancer, HER2 overexpression is significantly more frequent in tumors showing tubular histotype, high histological grade, advanced stage, and high Ki-67 labelling index, which suggests that it may represent a negative prognostic parameter [19].

In our study, similar to Fassan et al. [20], no relation was found between HER2/neu positivity and patients' age, gender. Also, we could not detect significant relation between Her2 expression and tumor location, tumor size and shape of the tumor which was in agreement with Chua et al. [21].

HER2/neu positivity in our series did not relate significantly with lymphovascular tumor emboli and perineural invasion which is in agreement with Chua et al. [21]. However, El-Gendi et al. [12] reported significant association between the HER2/neu positive status and lymphovascular emboli. and the pathologic T stage.

Conclusion

The rate of HER2/neu positivity in resectable gastric cancinomas was high (about 54%). The only statistical significant relation was found between positive Her2/neu expression and Lauren intestinal type. There was no association between HER2/neu positivity and the other clinical pathologic parameters.

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