RESEARCH ARTICLE

Frequency of Human Epidermal Growth Factor Receptor 2 (Her2/Neu) Expression in Gastric Adenocarcinoma in Rehman Medical Institute Peshawar

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Abstract

Objective: To investigate the frequency of Human Epidermal Growth Factor Receptor 2 over expression in gastric adenocarcinoma by immunohistochemistry and to find the association of its expression with clinicopathological parameters.

Methods: The descriptive cross-sectional study was conducted at Rehman Medical Institute, Peshawar, Pakistan, from January to December 2016, and comprised consecutive formalin-fixed and paraffin-embedded samples of gastric adenocarcinoma. The cases were scored for Human Epidermal Growth Factor Receptor 2 expression according to criteria cited in Trastuzumab for Gastric Cancer trial. Correlation of the expression with different clinicopathological parameters was determined. SPSS 23 was used for data analysis.

Results: Of the 55 cases, 49(89%) were biopsies and 6(11%) were gastrectomies. Among the patients whose samples were tested, 41(74.5%) were male. The overall mean age was 59.16±12.58 years (range: 38-95 years). Human Epidermal Growth Factor Receptor 2 overexpression (3+) was present in 19(34.5%) cases.Out of 21(38.2%) cases of moderately differentiated adenocarcinoma, 10(47.6%) showed overexpression. It was commonest in tumours of the fundus area 7(31.6%). No association of the expression was found with tumour's histological grade and location, or with patient's gender and age (p>0.05 each).

Conclusion: More than one-third of the sample had overexpression of Human Epidermal Growth Factor Receptor 2.

Keywords: Gastric carcinoma, HER2/neu, Immunohistochemistry, Fluorescence in situ hybridisation, Trastuzumab.
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Introduction

Worldwide, gastric carcinoma is the 4th commonest cancer and the 3rd commonest cause of cancer-related mortality. The 5-year survival rate is approximately 20%.[1] Helicobacter pylori (H. pylori) infection, atrophic gastritis, intestinal metaplasia and dysplasia are the risk factors for the development of gastric adenocarcinoma.[2]

Human Epidermal Growth Factor Receptor 2 (HER2/neu) is a proto-oncogene belonging to the tyrosine kinase receptor family. It has a major role in promoting cell proliferation and suppressing apoptosis.[3,4] In addition to breast carcinoma, HER2/neu is overexpressed in pulmonary adenocarcinoma, colorectal adenocarcinoma, pulmonary squamous cell carcinoma, gastric adenocarcinoma, prostatic adenocarcinoma and transitional cell carcinoma of urinary bladder.[4] In contrast to breast carcinoma, the effect of HER2/neu status on prognosis in gastric carcinoma remains contentious.[3] Just like in Her2/neu positive breast cancer, Trastuzumab (Herceptin) is also effective in Her2-positive gastric adenocarcinoma and is approved, in combination with chemotherapy, in the international phase III Trastuzumab for Gastric Cancer (ToGA) trial.[5] It can be continued as monotherapy in patients with chemotherapeutic adverse reactions.[3]

The frequency of HER2-positive gastric cancer varies from 6% to 29.5%.[3] The status of HER2/neu expression can be evaluated both on endoscopic biopsies or resection specimens using immunohistochemistory (IHC) or fluorescence in situ hybridisation (FISH).[3]

The current study was planned to determine HER/neu overexpression in gastric adenocarcinomas using IHC in a tertiary care setup. To the best of our knowledge this is the first such study from our region.

Materials and Methods

The descriptive, cross-sectional study was conducted at Rehman Medical Institute (RMI), Peshawar, Pakistan, from January to December 2016 after approval from the institutional ethics review board. Using medical record number of patients, all the data was retrieved from lab information system (LIS) database, maintaining the confidentiality of patients. Consecutive cases of gastric adenocarcinoma admitted to the hospital were included. Both endoscopic and gastrectomy specimens of adenocarcinoma were included. All tumours of the

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Table-1: Human Epidermal Growth Factor Receptor 2 (Her2/neu) scoring criteria by immunohistochemistry (IHC) in gastric and gastroesophageal junction adenocarcinoma.[5]

HER2 IHC Score	HER2 IHC Pattern in Surgical Specimen	HER2 IHC Pattern in Biopsy Specimen	HER2 Expression Assessment
0	No reactivity or membranous reactivity in <10% of cancer cells	No reactivity or no membranous reactivity in any cancer cell	Negative by IHC
1+	Faint or barely perceptible membranous reactivity in ≥10% of cancer cells; cells are reactive only in part of their membrane	Cancer cell cluster* with a faint or barely perceptible membranous reactivity irrespective of percentage of cancer cells positive	Negative by IHC
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Cancer cell cluster* with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of cancer cells positive	Equivocal by IHC
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of cancer cells	Cancer cell cluster* with a strong complete basolateral, or lateral membranous reactivity irrespective of percentage of cancer cells positive	Positive by IHC

* Cancer cell cluster consisting of ?5 neoplastic cells.

stomach other than adenocarcinoma, e.g. squamous cell carcinoma, adenosquamous carcinoma, mixed adenoneuroendocrine carcinoma, high-grade neuroendocrine carcinoma and undifferentiated carcinoma, were excluded.

Guidelines of the College of American Pathologists were followed for gross examination of resection specimens, using 10% neutral buffered formalin for fixation.[6] For light microscopy, 4 micron sections of formalin-fixed and paraffin-embedded (FFPE) tissue blocks were stained with haematoxylin and eosin(H&E). Diagnosis was made on H&E slides, and tumours were graded histologically based on the extent of glandular differentiation. FFPE tissue blocks were used for determining HER2/neu status using Polyclonal Rabbit Anti-Human antibody (Dako, Agilent, USA).

FFPE block of known breast carcinoma with strong positive HER2/neu was used as positive control. HER2/neu expression was scored from 0 to 3+ independently by two senior pathologists using ToGA criteria (Table-1). Tumours having IHC scores of 0 or 1+ were considered negative for HER2 overexpression, whereas tumours scoring 3+ were considered positive. IHC score of 2+ was considered equivocal. In endoscopic biopsies, location of the tumour was confirmed by a gastroenterologist. Statistical analysis was carried out using SPSS23. Chi square test was used to determine the association between HER2/neu overexpression and clinicopathological parameters. P<0.05 was considered statistically significant.

Results

Of the 55 cases, 49(89%) were biopsies and 6(11%) were

Table-2: Human Epidermal Growth Factor Receptor 2 (Her2/neu) expression in various clinicopathological variables.

Clinico-pathologic Variables	HER2				P value
	0	1+	2+	3+	
AGE					0.19
>=60 yrs (n=29)	0 (0.0%)	9 (31%)	9 (31%)	11(37.9%)	
< 60 yrs (n=26)	2 (7%)	4 (15.4%)	12 (46.1%)	8 (30.8%)	
GENDER					0.60
Male (n=41)	2 (4.8 %)	11 (26.8%)	15 (36.5%)	13 (31.7%)	
Female (n=14)	0 (0.0%)	2 (14.3%)	6 (42.9%)	6 (42.9%)	
GRADE					0.16
Well differentiated (n=19)	0 (0.0%)	4 (21%)	8 (42%)	7 (36.8%)	
Moderately differentiated (n=21)	2 (9.5%)	4 (19%)	5 (23.8%)	10 (47.6%)	
Poorly differentiated (n=15)	0 (0.0%)	5 (33.3%)	8 (53.3%)	2 (13.3%)	
LOCATION					0.08
Cardia (n=4)	0(0.0%)	2(50%)	2(50%)	0(0.0%)	
Fundus (n=8)	0(0.0%)	0(0.0%)	2(25%)	6(75%)	
Body (n=19)	2(10.5%)	7(38.8%)	5(26.3%)	5(26.3%)	
Antrum (n=10)	0(0.0%)	0(0.0%)	6(60%)	4(40%)	
Gastro-esophageal junction (n=14)	0(0.0%)	4(28.6%)	6(42.8%)	4(28.6%)	

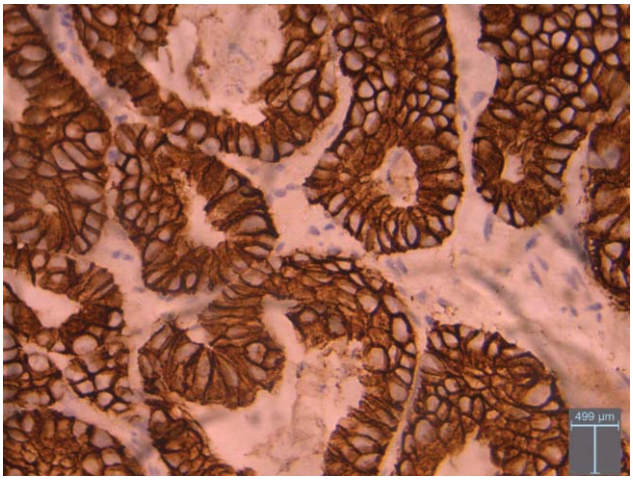

Figure-1: Human Epidermal Growth Factor Receptor 2 (Her2/neu) immunohistochemistry (IHC) stain at 40x magnification showing 3+ positivity.

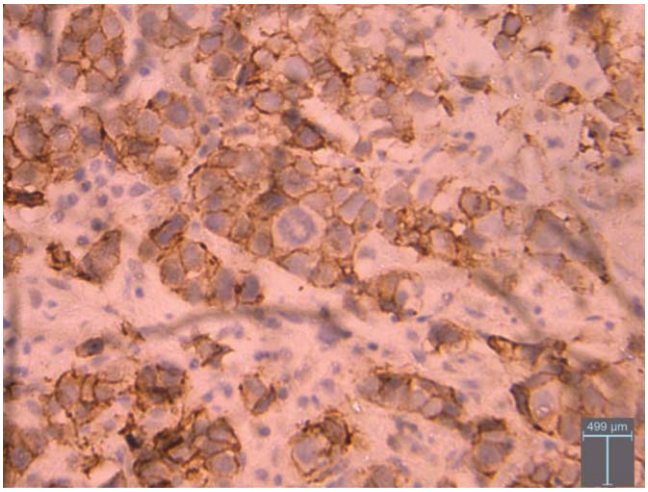

Figure-2: Human Epidermal Growth Factor Receptor 2 (Her2/neu) immunohistochemistry (IHC) stain at 40x magnification showing 2+ (equivocal) positivity.

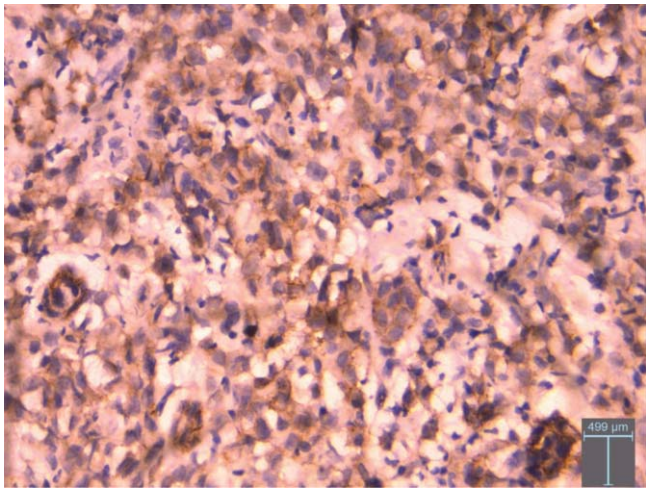

Figure-3: Human Epidermal Growth Factor Receptor 2 (Her2/neu) immunohistochemistry (IHC) stain at 40x magnification showing barely perceptible membranous staining (1+ score).

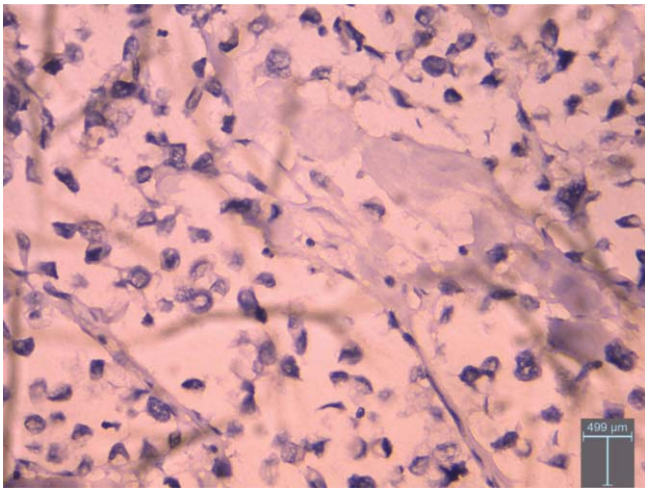

Figure-4: Human Epidermal Growth Factor Receptor 2 (Her2/neu) immunohistochemistry (IHC) stain at 40x magnification showing no reactivity (score 0).

gastrectomies. Among the patients whose samples were tested, 41(74.5%) were male. The overall mean age was 59.16±12.58 years (range: 38-95 years). Majority 24(43.6%) patients were in the 60-70 years age group. Body of stomach was the commonest location of tumour 18(32.7%), followed by gastroesophageal junction 14(25.5%). Overall, 21(38.2%) tumours were moderately differentiated, followed by 19(34.5%) well differentiated adenocarcinoma.

A score of 2+ (equivocal) was seen in 20(36.4%) cases, followed by 3+ in 19(34.5%) (Figures 1-2). HER2/neu-negative cases (0 and 1+) collectively constituted 16(29.1%) cases (Figures 3-4). HER2 positivity (3+) was more in females 6(42.8%) compared to males 13(31.7%). Moderately differentiated and well differentiated tumours expressed 3+ positivity in 10(47.6%) and 7(36.8%) cases respectively. Of the total 19(34.5%) cases of HER2/neu 3+positivity, 7(31.6 %) were of fundus origin. None of the 6(11%) gastrectomy specimens were HER2/neu-positive. No association was found between HER2/neu positivity and histological grade (p=0.16). Similarly, there was only a trend towards an association between HER2 positivity and site of tumour (p=0.08). HER2/neu scores of 0, 1+,2+and 3+ in different histological grades, tumour locations, age groups and gender were noted separately (Table-2).

Discussion

Hippocrates was the first to use the words 'Karkinos; and 'Karkinoma' in Greek for cancer and carcinoma.[7] Probable gastric carcinoma cases were first reported in Ebers Papyrus, an Egyptian compilation of medical knowledge, that was written in 1600 BC.[7] J. Cruveilhier was the first to describe benign and malignant gastric ulcers in 1835AD which explained the enigma behind the death of Napoleon Bonaparte.[7]

Incidence of gastric cancer varies, with highest rates reported in eastern Asia, eastern Europe and South America; and lowest rates in North America and Africa.[8] In Pakistan, the incidence is somewhat lower than the neighbouring countries and most of the patients present in the 7th decade of life.[9] In our study, 43.6% patients were in the 6th decade. Statistics from Hazara Division showed lowest prevalence (2.70%) in all age groups and both genders combined.[10] Prevalence in Karachi was found to be 6 per 100,000 in males and 3.6 per 100,000 in females.[11]

Hofmann et al. assessed the scoring system for HER2/neu that was used in breast carcinoma to determine HER2/neu positivity in 178 resection specimens of gastric adenocarcinoma. He concluded that a modified scoring system for HER2 expression is needed in gastric adenocarcinoma.[12] After the approval of Trastuzumab, a monoclonal antibody against HER2/neu, as first-line treatment in combination with chemotherapy for HER2-positive advanced gastric or gastro-oesophageal junction cancers, pathologists now routinely assess HER2/neu expression by IHC followed by FISH in HER2/neu 2+ cases in gastric and gastro-oesophageal adenocarcinoma by the ToGA criteria.[13]

Researchers from various geographical zones determined HER2/neu expression in gastric adenocarcinoma and found marked variability in its expression.[14] Prevalence of HER2 expression in gastric adenocarcinoma ranges from 6% to 29.5%.[3] Higher expression of 35.89% and 44.2% is noted in two studies conducted in Indian population.[15,16] Our study population showed HER2/neu 3+ expression in 34.5% cases. We did not find any similar study done in our country except one which determined HER2/neu expression in 50 cases of adenocarcinoma of the whole gastrointestinal (GI) tract. Out of 50 cases, only 11 were of gastric adenocarcinoma and out of these 11 cases, 2(18%) showed HER2 3+ positivity while 6 cases were equivocal.[17] Comparison of Her2/neu expression in our study with studies conducted in other geographical zones, including China, United States, Italy, Egypt, India, Korea, Germany, Iran, Brazil are interesting (Table 3).

In our study, 47.6 % cases of moderately differentiated adenocarcinoma showed HER2 3+ positivity, followed by

Table-3: Frequency of Human Epidermal Growth Factor Receptor 2 (Her2/neu) expression in gastric adenocarcinoma by immunohistochemistry (IHC) and fluorescence in situ hybridization/ chromogenic in situ hybridization (FISH/CISH) in various geographic zones.

Author (year)	Country	Total number of cases	Technique	HER2 Frequency in Gastric adenocarcinoma	
				Protein expression (HER2 3+)	Gene amplification
Hofmann et al. (2008)[11]	Germany, China, Mexico	168	IHC & FISH	10.7%	17.4%
Lakshmi et al. (2014)[14]	India	98	IHC	37.9%	---
Sekaran et al. (2012)[15]	India	52	IHC & FISH	44.2%*1	---
Rajagopal et al. (2015)[17]	India	60	IHC	26.7%	---
Shan et al. (2013)[18]	China	1463	IHC & FISH	9.8%	17.2%
Abdel Hadi et al. (2016)[19]	Egypt	85	IHC & FISH	14.2%	25.9%
Ansari et al. (2011)[20]	Iran	100	IHC	7%	---
Madani et al. (2015)[21]	Iran	211	IHC	11.8%	---
Tewari et al. (2013)[22]	India	70	IHC	21.4%*	---
Badary et al. (2017)[23]	Egypt	42	IHC	59.5%	---
Park et al (2006)[26]	Korea	182	IHC, FISH& CISH	6%	3.8%
Rakhshani et al. (2014)[27]	Iran	101	IHC & CISH	12.9%	16.8%
Son et al. (2014)[28]	Korea	139	IHC & FISH	15.1%*1	---
Abdel-Aziz et al. (2017)[29]	Egypt	48	IHC	22.9%	---
De Carli et al. (2015)[30]	Brazil	48	IHC	14.5%	---
Tafe et al. (2011)[31]	USA	135	IHC & FISH	15.5%	14.8%
Our study	Pakistan	55	IHC	34.5%	---

*Both 2+ and 3+ cases were considered positive.

*1 FISH was performed for IHC 2+ cases, so it includes both IHC 3+ cases and those 2+ cases which came positive on FISH.

36.8% of well-differentiated cases. In a study of 52 cases of gastric carcinoma in Indian population, HER2/neu3+ positivity, like our study, was commonest in moderately differentiated tumours (53%), followed by poorly differentiated adenocarcinoma (39%).[16] Rajagopal et al. also found HER2/neu 3+ positivity most commonly in moderately differentiated adenocarcinoma (37.5%). Well-differentiated cases showed 11.1% positivity, while none out of 11 poorly differentiated cases showed 3+ positivity.[18] In a Chinese study of 1463 patients, HER2/neu 3+ positivity was most common in moderately differentiated cases (20.1%), followed by well-differentiated adenocarcinoma (16%).[19] Moderately differentiated gastric adenocarcinoma was also the commonest (18%) to express HER 3+ positivity in an Egyptian study.[20] In these three studies, like our study, HER2 3+ positivity was most commonly seen in moderately differentiated adenocarcinoma.

In a study, 60% of well-differentiated cases showed 3+ positivity, followed by moderately differentiated cases (29.1%).[15] An Iranian study on 100 cases of gastric adenocarcinoma showed 3+ positivity in 19.6% and 18.1% of well- and moderately differentiated cases respectively. Poorly differentiated carcinomas were all HER2-negative.[21] Madani et al. found well-differentiated cases (17.5%) to be the commonest in expressing HER2 3+ positivity followed by moderately differentiated cases (11.7%).[22] Hence, in contrast to our study, in these three studies, well-differentiated adenocarcinomas were the commonest to express HER2 3+ positivity.

We found no association between HER2/neu overexpression and tumour differentiation/grade (p=0.16). This is also supported by other studies.[16,20,21,23-25] Jeung et al. also did not find any statistical correlation between the two variables in 116 cases of gastro-oesophageal adenocarcinoma (gastric 54 cases, oesophageal/gastro-oesophageal junction 62 cases).[26] Park et al also found no correlation between HER2 gene amplification determined by FISH and histological grade.[27] In contrast, several studies found significant correlation between HER2 3+ expression and tumour grade.[18,19,22,23,28-30]

In our study, body of the stomach was the commonest tumour location (32.7%), followed by gastro-oesophageal junction (25.5%), while HER2 3+ positivity was most commonly seen in tumours of fundus area (31.6%). Abdel-Aziz et al. found antral tumours to be the commonest both in terms of location (41.7%) and HER2 3+ expression (30%).[30] In a study, distal stomach was the commonest location (87.5%) and HER2 3+ positivity was more in proximal stomach tumours (16.6%).[31] Son et al. also found distal stomach to be the commonest location (54.6%), followed by middle stomach in 28.8% cases. HER2/neu positivity was commonest in proximal stomach (22.7%).[29] Antrum was the commonest tumour location (76.7%) followed by gastro-oesophageal junction in a study[18] but it did not document Her2/neu positivity in different tumour locations.[18]

In our study, there was only a trend towards association between HER2/neu overexpression and location of tumour which is supported by another study.[30] Similarly, HER2/neu overexpression was not influenced by tumour site in several studies.[29-31] In our study, gender and age were not found to have significant correlation with HER2/neu overexpression, which is supported by other studies.[18,19,22,24,27,28,30]

The important limitation of our study was that IHC 2+ and 1+ cases were not confirmed by FISH testing due to unavailability of the facility. As the IHC-FISH concordance is 97% and 100% in IHC 0 and 3+ cases respectively, so their confirmation with FISH is not generally required.[32] Another limitation was that correlation of HER2/neu expression with tumour stage and lymph node metastasis could not be determined because of small sample size of gastrectomy specimens (6/55 cases), although all of our gastrectomy cases were HER2/neu-negative.

A larger study consisting of resection (gastrectomy) specimens and FISH testing in IHC 2+ cases will give more accurate estimate of HER2/neu positivity in gastric adenocarcinoma.

Conclusion

For the selection of patients who may benefit from Trastuzumab, an accurate evaluation of HER2/neu is of prime importance. We found HER2/neu 3+ expression by IHC in 34.5% cases. This number may even be greater if all HER2/neu 2+ cases were also subjected to FISH testing. Hence, a larger number of patients in our region would benefit from Trastuzumab.

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Conflict of Interest: None.

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