Original Article

Gastric adenocarcinoma expressing human epidermal growth factor receptor in South Asian population

Asma Shabbir, Muhammad Asif Qureshi[1], Abdullah Bin Khalid[1], Talat Mirza[2], Asma Shaikh, Syed Mehmood Hasan

Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, [1]Department of Pathology, Dow International Medical College, Dow University of Health Sciences, [2]Department of Pathology, Doctor. Ziauddin Hospital and University, Karachi, Pakistan

Abstract

Background/Aim: Gastric cancer is the third leading cause of cancer mortality worldwide. Human epidermal growth factor (Her-2/*neu*) has shown strong therapeutic implication in breast cancer. Although the prevalence of Her-2/*neu* over-expression in gastric cancer has been reported across the world, it is still unknown from South Asia. The aim of this study is to evaluate Her-2/*neu* expression in gastric adenocarcinomas and to correlate with various clinicopathological variables.

Patients and Methods: A total of 95 consecutive patients undergoing endoscopic biopsy or gastrectomy were recruited in this study. Clinicopathological parameters of all patients were recorded and hematoxylin and eosin (H and E) staining was performed. Over-expression of Her-2/*neu* was investigated by immunohistochemistry using α-Her-2 antibody. To quantify Her-2/*neu* over-expression, the Hofmann validation scoring system was used and further its association was seen with age, gender, histopathological type, grade, and stage of the tumor. Data were entered and analyzed using SPSS version 21. A *P* value of <0.05 was considered as significant.

Results: Overall, 21 (22.1%) cases were positive for Her-2/*neu* overexpression from the total of 95 gastric adenocarcinomas. Her-2/*neu* was significantly expressed in low-grade gastric cancer (grade I = 50%, grade II = 34.5%, grade III = 14.5%; $P = 0.030$). Although there was insignificant difference between Her-2/*neu* over expression and other variables, Her-2/*neu* score 3+ was predominantly seen in females, age >60 years, Laurens intestinal type, and IIIC stage tumors.

Conclusion: Her-2/*neu* is over-expressed in a limited group of gastric cancer patients in our population and indicates a significant strong association with low grades of gastric cancer.

Keywords: Gastric adenocarcinoma, Her-2/*neu*, immunohistochemistry

Address for correspondence: Dr. Asma Shabbir, Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan.
E-mail: drasma52@gmail.com

INTRODUCTION

Gastrointestinal malignancies are among the major oncological problems worldwide. Gastric cancer is the fifth most common cancer and third leading cause of death across the globe.[1] South Asia is a low to moderate risk region for gastric cancer.[2] In Pakistan, although a lower incidence is reported, the mortality rate due to gastric cancer appears to be high (13.3%).[3] Most of the patients present with advanced stages, and thus the survival of

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the patients remain poor with palliative chemotherapy.[4,5] Hence, newer therapeutic targets are desired to improve the survival rate.

The human epidermal growth factor (Her-2/neu) is a proto-oncogene located on chromosome 17q21 that encodes for ErbB-2 and is believed to play a significant role in tumorigenesis of gastric cancer.[6,7] Over-expression of Her-2/neu has been notably associated with increased cellular survival, increased proliferation, and decreased apoptotic potential of cells leading to malignant transformation and maintenance of the associated malignancy.[8] The presence of Her-2/neu has been strongly implicated in certain cancers including lungs, colon, esophagus, bladder, and head and neck.[9] Her-2/neu is overexpressed in about 20%–30% of breast cancer, and use of trastuzumab, a monoclonal antibody against Her-2/neu, has shown a good prognosis in these patients.[10]

There are now increasing data available to indicate over-expression of Her-2/neu in patients with gastric adenocarcinomas. Recently, a phase III trial has been conducted using targeted therapy by trastuzumab against Her-2/neu which has shown improved survival in metastatic gastric adenocarcinoma patients.[6,11] This has rapidly increased the demand for Her-2/neu assessment in gastric cancer patients.[12] Moreover, high concordance rate of Her-2/neu overexpression in primary and secondary gastric cancer sites suggests evaluation of Her-2/neu in primary gastric cancer as a reliable basis to determine anti Her-2 therapy in metastatic gastric cancer patients.[13] However, and alarmingly, data regarding overexpression of Her-2/neu in patients with gastric cancer in South Asia region are very scarce.[4,7] Therefore, in this study we aimed to investigate the frequency of Her-2/neu over-expression in Pakistani patients with gastric adenocarcinoma.

PATIENTS AND METHODS

Recruitment of patients and ethical approval

The study was conducted at the Dow Diagnostic Research and Reference Laboratory (DDRRL), Dow University of Health Sciences (DUHS), Karachi, after ethical approval of Institutional Review Board, between May 2014 and February 2016. A total of 95 consecutive patients with gastric carcinoma who were planned to undergo biopsy at National Institute of Liver and Gastrointestinal Diseases (NILGID) and gastrectomy at surgical ward of DUHS were recruited in the study after their informed consent. Clinicopathological parameters of the patients were then recorded.

Processing of tissues and microscopy

Subsequent to biopsy/gastrectomy, specimens were transferred to histology section in 10% neutral buffer formalin. Samples were examined for gross features, and paraffin blocks were prepared for subsequent staining and microscopy. Tissue sections, each measuring 3–4 μm in thickness were cut from paraffin blocks and processed for hematoxylin and eosin (H and E) staining. Based on microscopic examination of the H and E-stained slides, clinicopathological parameters were recorded including tumor type and tumor grade among others. Histopathological grading of tumors was performed according to the World Health Organization (WHO) criteria as grade I (well-differentiated), grade II (moderately differentiated), and grade III (poorly differentiated). Pathological staging of gastrectomy cases was recorded as per the seventh edition of the American Joint Committee on Cancer Staging (stage I–stage IV).[14]

Immunohistochemistry and scoring of Her-2/neu overexpression

To investigate over-expression of Her-2/neu in gastric adenocarcinoma, conventional immunohistochemistry protocol was subjected on all cases. Sections were deparaffinized and dehydrated, and antigen retrieval was performed. Furthermore, the sections were incubated with mouse primary monoclonal antibody against Her-2/neu (clone CB-11, dilution 1:65; Cell Marque) for 1 h in moisturization chamber. Secondary antibody (HiDef Detection System) (Cell Marque) containing solution A as amplifier and solution B as polymer was used. For all read-outs, staining was controlled using known Her-2/neu overexpressing breast carcinoma tissues.

Her-2/neu-stained slides were independently evaluated by two experienced pathologists. The scoring was performed according to Hofmann Validation Criteria taking into account 10% of tumor cells for resection specimens and tumor cell cluster (neoplastic cells, irrespective of percentage of tumor cells stained) for biopsy specimens as cut-off value for the expression of Her-2/neu. 0/−ve for no staining in <10%/tumor cell cluster, 1+/−ve for faint/barely perceptible basolateral membrane staining >10%/tumor cell cluster, 2+/equivocal for weak to moderate complete membrane or basolateral membranous staining in >10%/tumor cell cluster, and 3+/positive for strong complete membrane or basolateral membranous staining in >10%/tumor cell cluster [Table 1].[15,16] Scores 0 and 1+ were considered as negative, score 2+ as equivocal, and score 3+ as positive.

Statistical analysis

Data were recorded for different variables including age and gender of the patients, histopathological type, grade,

stage of the tumor, and Her-2/neu expression. This was followed by entry and analysis using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA version 21). To investigate association of Her-2/neu over-expression in gastric adenocarcinomas with other categorical variables, various statistical tests including Chi-square (when expected value of the cells was >5) and Fisher's exact test (when expected value of the cells was <5) were applied. A *P* value <0.05 was considered as significant.

RESULTS

Clinicopathological parameters of gastric cancer patients

The study included a total of 95 cases of gastric adenocarcinoma. The median age of the patients recruited in this study was 51 years (range 22–85 years). Of all the patients studied, a total of 78 (82%) were males, whereas 17 (17.9%) were females. A total of 78 (82.1%) were biopsy specimens, whereas 17 (17.9%) were gastrectomy specimens. Overall, a total of 4 (4.2%) patients had grade I, 29 (30.5%) had grade II, and 62 (65.3%) had grade III tumors. Histologically, 35 (36.8%) tumors were Lauren's intestinal, 39 (41.1%) were diffuse, and 21 (22.1%) were mixed variants [Table 2]. Pathological staging could only be performed on gastrectomy samples ($n = 17$) with stage IIA being the most frequent in our study group [Table 3].

Her-2/neu immunoreactivity in gastric cancer

Of the total 95 gastric cancer tissues, 26 (27.3%) cases were scored as 0/−ve, 21 (22.1%) were scored as 1+/−ve, 27 (28.4%) cases as 2+/equivocal, and 21 (22.1%) cases as 3+/positive for Her-2/neu staining intensity [Figures 1-4]. Overall, Her-2 score 3+ was significantly higher in low-grade gastric cancer (50% in low grade vs 14.5% in high grade, $P = 0.030$). Although there was no significant difference between Her-2/neu overexpression and other variables, Her-2/neu score 3+ was higher in females ($P = 0.714$), age group <60 years ($P = 0.636$), Lauren's intestinal type ($P = 0.053$), and *P* IIIC stages ($P = 0.239$) [Table 4].

Table 1: Hofmann validation criteria 15

Pattern of Staining	% of tumor cells stained	Score
No staining	<10%/tumor cell cluster*	0/-ve
Faint/barely perceptible Basolateral membrane staining	>10%/tumor cell cluster*	1/-ve
Weak to moderate complete membrane/ Basolateral membrane staining	>10%/tumor cell cluster*	2+/equivocal
Strong complete membrane/ Basolateral membrane staining	>10%/tumor cell cluster*	3+/+ve

*Tumor cell cluster = ≥5 neoplastic cells

Table 2: Clinicopathological characterization of gastric cancer cases

Variable/parameter	*n* (%)
Age	
<60 years	63 (66.3)
≥60 years	32 (33.7)
Gender	
Male	78 (82.1)
Female	17 (17.9)
Grade	
I	4 (4.2)
II	29 (30.5)
III	62 (65.35)
Histological type	
Intestinal	35 (36.8)
Diffuse	39 (41.1)
Mixed	21 (22.1)
Specimen	
Endoscopic biopsy	78 (82.1)
Gastrectomy	17 (17.9)
Total (*n*)	95

Table 3: Frequency of pathological stage in gastrectomy specimen

Pathological stage	*n* (%)
I	0
IIA	8 (47.1)
IIB	2 (11.8)
IIIA	1 (5.9)
IIIB	5 (29.4)
IIIC	1 (5.9)
Total (*n*)	17

Note: Pathological staging could only be determined in gastrectomy specimen ($n=17$)

DISCUSSION

In this study, we have investigated Her-2/neu overexpression in Pakistani patients with gastric adenocarcinoma using immunohistochemistry. Literature shows the overexpression of Her-2/neu in gastric carcinomas to be extremely variable ranging from 7% to 43% positivity.[17] In this study, Her-2/neu overexpression was found in 22.1% of cases which is at lower rate when compared with another study done in the same region where Her-2/neu overexpression was 90%.[17] There could be several reasons for this discrepancy. First, the other study done in the same region investigated a smaller sample than ours. Moreover, they considered scores 1+, 2+, and 3+ as positive for Her-2/neu overexpression, whereas we strictly followed the Hofmann scoring protocol with only 3+ score as positive. In addition, our reporting protocol is consistent with the current standards of Her-2/neu interpretation based on National Comprehensive Cancer Network (NCCN). A study conducted by Allgayer *et al.* among 203 cases of gastric cancer also reported a high rate (91%) of positivity of Her-2/neu overexpression.[18] Here, they considered both membranous and cytoplasmic staining as positive for Her-2/neu overexpression, and this might have given

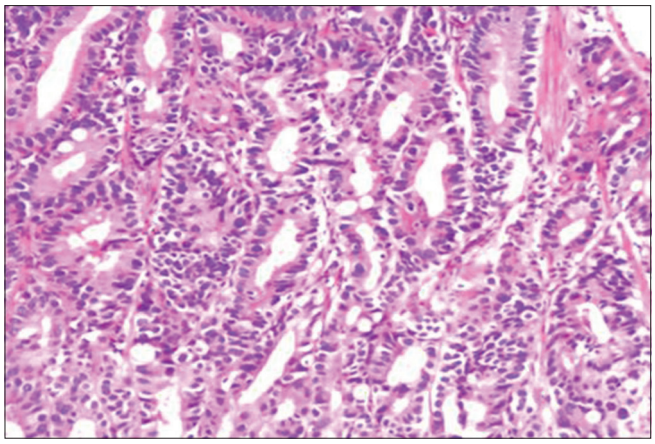

Figure 1: H and E stain grade I gastric cancer

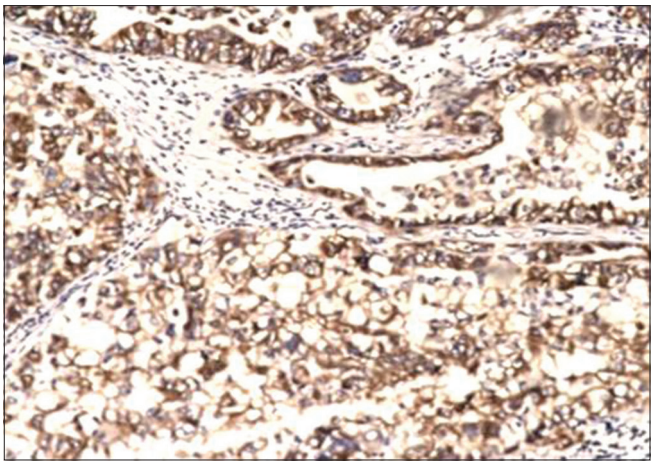

Figure 2: Her-2/*neu* score 3+ in grade I gastric cancer

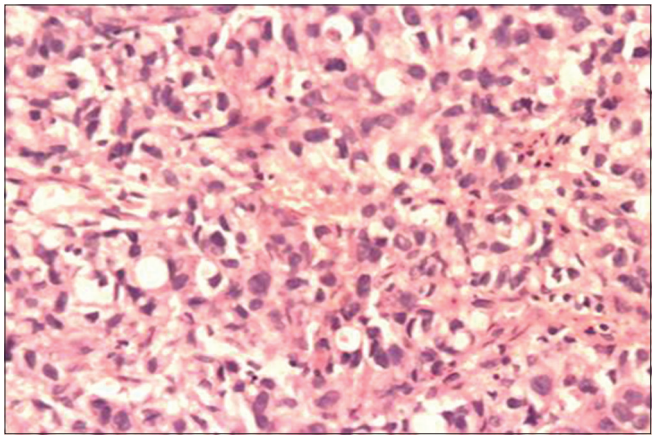

Figure 3: H and E stain grade III gastric cancer

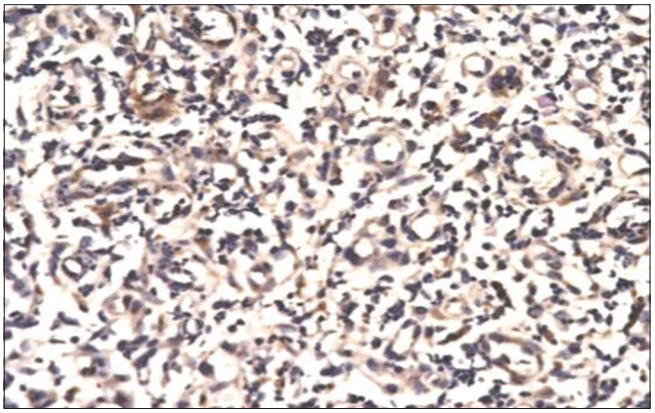

Figure 4: Her-2/*neu* score 2+ in grade III gastric cancer

Table 4: Expression of Her-2/*neu* in gastric cancer

Variable/ parameter	n	Her-2/*neu* score				*P*
		0	1+	2+	3+	
Gender						
F	17	5 (29.4)	5 (29.4)	3 (17.6)	4 (23.5)	**0.714
M	78	21 (26.9)	16 (20.5)	24 (30.8)	17 (21.8)	
Age						
≤ 60 yrs	63	19 (30.2)	12 (19.1)	17 (27.0)	15 (23.8)	*0.636
≥ 60 yrs	32	7 (21.9)	9 (28.1)	10 (31.3)	6 (18.8)	
Grade						
I	4	0	2 (50.0)	0	2 (50.0)	**0.030
II	29	10 (34.5)	3 (10.3)	6 (20.7)	10 (34.5)	
III	62	16 (25.8)	16 (25.8)	21 (33.9)	9 (14.5)	
Type						
Intestinal	35	13 (37.1)	5 (14.3)	5 (14.3)	12 (34.3)	*0.053
Diffuse	39	10 (25.6)	9 (23.1)	14 (35.9)	6 (15.4)	
Mixed	21	3 (14.3)	7 (33.3)	8 (38.1)	3 (14.3)	
pTNM						
IIA	8	3 (37.5)	4 (50.0)	1 (12.5)	0	**0.239
IIB	2	1 (50.0)	1 (50.0)	0	0	
IIIA	1	0	0	1 (100)	0	
IIIB	5	2 (40.0)	1 (20.0)	0	2 (40.0)	
IIIC	1	0	0	0	1 (100)	

* Pearson Chi square .** Fisher's exact, level of significance at 0.05

higher positive rates of Her-2/neu overexpression in their series. These variable data could be attributed to several factors including use of different antibodies, different sample size, and use of non-uniform scoring system for interpretation of results among others.[19] However, Her-2/neu overexpression in our study is found in limited population which is in accordance with few other studies reported from South Asia region using the same scoring criteria.[4] One of the shortcoming of our study was the lack of confirmatory fluorescent *in situ* hybridization (FISH) test for score 2+ cases. Although there exists a debate for testing Her-2/neu overexpression preferably through FISH, the concordance rate remains 95%, indicating that both techniques generate equally reproducible results for score 3+ cases.[20]

The published literature shows a diverse relationship between Her-2/neu overexpression in gastric cancer with various clinicopathological factors.[21-24] Our series comprised less number of low-grade gastric cancer cases which might be due to gastric cancer patients presenting at advanced stages to the hospital.[3] However, in agreement with other studies, we noted higher positivity of Her-2/neu overexpression in low grade of gastric cancer, suggesting an early molecular event in oncogenesis.[20,21,25-28] Our study observed insignificant association of Her-2/

neu overexpression with age, gender, type, and stage of gastric cancer which is consistent with other studies.[4,7,20,29] Nevertheless, our study included a relatively larger cohort of patients when compared with the other reported studies from the South Asia region which have used a limited number of samples to elucidate their findings.

We conclude that Her-2/neu is overexpressed in a limited group in our population. It is significantly overexpressed in low-grade gastric carcinoma, and overexpression is not dependent on various other clinicopathological parameters. Furthermore, to avoid the inconsistency in results and for better reporting of Her-2/neu overexpression in gastric carcinoma, we suggest to follow a standardized protocol.

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Conflicts of interest

There are no conflicts of interest.

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