HER2 Expression in Gastric and Gastroesophageal Cancer: Report from a Tertiary Care Hospital in North India

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Abstract Despite improvements in chemotherapy, survival of metastatic gastric and gastroesophageal junction (GEJ) adenocarcinoma remains poor. Trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), has shown promise in improving survival of these patients by a recent large phase III trial. HER2 status in gastric and GEJ cancers, although reported from across the world, is yet unknown in India due to lack of published literature from the country. HER2 status in 70 samples of gastric and GEJ adenocarcinomas (Siewert type III) was evaluated by immunohistochemistry (IHC) in this study using the gastric cancer scoring system. It was also correlated with clinic–pathologic factors. Samples with IHC score 2+ and 3+ were taken as HER2 positive. HER2 overexpression was found in 15 (21.4 %) samples, was significantly ($p=0.006$) more common in intestinal type (45 %), but it did not correlate with age, gender, stage, or grade of tumor and did not affect the 2-year disease-free survival. HER2 overexpression is found only in a minority of patients with gastric and GEJ cancers in the Indian population. A large cohort of patients with a longer follow-up will be

required to assess for any significant statistical association of HER2 expression with prognosis of these patients.

Keywords Gastric cancer · Gastroesophageal cancer · HER2 expression

Introduction

Cancer of the stomach and gastroesophageal junction (GEJ) constitutes a major health problem worldwide. It is the most common cancer diagnosed in men in Japan. Its incidence in the USA is, however, low [1]. The incidence of gastric cancer is decreasing and lies between 10 and 15 new cases per 100,000 population per year in most Western countries. Peak age is between 60 and 80 years. Whereas distal gastric cancers account for the overall decrease in gastric cancer, tumors in the proximal stomach (cardia and GEJ) are on the rise [2, 3]. In India, the incidence of gastric cancer is low, compared to the high-risk areas such as Japan, Korea, and China. The incidence rates vary from 13.56 per 100,000 (Chennai) in the southern parts to 57.3 among males and 33.6 among females from north–east India (Aizawl, Mizoram) [4].

Gastric cancer is often diagnosed in a locally advanced or metastatic stage when the tumor is unresectable. The current chemotherapeutic agents do not offer a substantial response with response rates varying from 10 to 60 %, and thus, the survival of patients on palliative chemotherapy remains poor [5]. Hence, there has been a tremendous effort for the search of new therapeutic strategies and targets. One such strategy is targeting the human epidermal growth factor receptor 2 (HER2), also known as *HER-neu*. The HER2 gene is a member of the epidermal growth factor receptor family. HER2 is encoded by a gene located on chromosome 17q21 [6]. The HER2 gene is located adjacent to the

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topoisomerase IIa genes. It encodes for the 185-kDa receptor, HER2, which has an extracellular ligand-binding domain, transmembrane and intracellular domain with tyrosine kinase activity. HER2 does not bind to any known ligand, but it forms a heterodimer with other members of the HER family and initiates a cascade of reactions that can influence many aspects of tumor cell biology, including cell proliferation, apoptosis, adhesion, migration, and differentiation. In carcinomas, HER2 acts as an oncogene [7]. High-level amplification of the gene induces protein overexpression in the cellular membrane and can be picked up by the fluorescence in situ hybridization (FISH) test.

HER2 has been found overexpressed in a number of neoplasm, the most studied being breast cancer where overexpression is reported in 10 to 34 % of cases [8]. Trastuzumab, a fully humanized monoclonal antibody, targets the extracellular domain of the HER2 receptor and thereby prevents its activation. Trastuzumab is already widely used for the treatment of HER2-positive breast cancer and has shown to improve survival in patients with HER-positive breast cancer [9]. This has encouraged investigation of its antitumor activity in patients with HER2-positive gastric adenocarcinomas.

The expression of HER2 in gastric cancer remains low. HER2 overexpression has been reported in 9.5 to 12 % of gastric cancer and 24 to 25 % GEJ cancer in various studies [10, 11]. In a recent trial, Trastuzumab for GAstric cancer (ToGA), patients assigned to the trastuzumab treatment protocol showed an improved overall survival over those not receiving this treatment. Median overall survival was reported as 13.8 months [95 % confidence interval (CI) 12–16] in those assigned to trastuzumab plus chemotherapy ($n = 294$) compared with 11.1 months (10–13) in those assigned to chemotherapy alone ($n = 290$) (hazard ratio 0.74; 95 % CI 0.60–0.91; $p = 0.0046$) [12]. Results of the ToGA trial have been very encouraging, opening a new channel of hope for the treatment of advanced gastric cancer. A subgroup analyses on 101 Japanese patients enrolled into ToGA study also revealed encouraging results [13].

Although data regarding frequency of HER2 expression have been reported from several countries across the globe, published data on Indian population are very limited [14, 15]. We hereby present our data on HER2 expression in gastric and GEJ cancer (Siewert type III).

Methods

Setting

The present study was carried out by the Department of Surgical Oncology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., a tertiary care government

referral hospital and medical institute in northern India. Clinical details, histopathology, and HER2 expression by immunohistochemistry (IHC) were evaluated in 70 gastric and gastroesophageal adenocarcinoma (Siewert type III) samples between January 2010 and January 2012. Forty-five samples were taken from the main specimen after radical gastrectomy and 25 were biopsy samples. The pathologist examining the slides was blind with regards to the clinical details and survival of the patients and was not involved in the statistical analysis and computation of results.

Immunohistochemical Analysis

The criteria used were as described by the ToGA trial and adopted by the National Comprehensive Cancer Network guidelines version 2.2011 [1, 12]. The criteria for biopsy samples were accordingly modified.

The gastric cancer tissue sample was fixed in 10 % neutral buffered formalin for 24 to 48 h and embedded in paraffin. Serial sections of 4 μ m thickness were obtained from tissue blocks and processed for immunohistochemical studies. Slides were then deparaffinized and rehydrated with descending grades of ethyl alcohol. IHC staining was done using the HER2 (BioGenex pre-diluted, Clone CB11, Mouse IgG) antibody.

Immunostaining was carried out with a manual method using a biotin–streptavidin complex. 3,3-Diaminobenzidine tetrahydrochloride was used as chromogen, and sections were counterstained with hematoxylin. Microwave pretreatment (slides were immersed in a 10-mM citrated buffer, pH 6.0, at 95 °C, 5 min $\times$ 3) for antigen retrieval was carried out prior to incubation with primary antibody. Positive tissue controls of the gastric carcinoma as well as negative control slides that were run simultaneously were used to assess the quality of immunostaining. Immunoreactivity was quantified by evaluating a minimum of 1,000 carcinoma cells in randomly selected fields on histologic sections, using a high-powered objective lens.

For the evaluation of HER2 expression, semi-quantitative analysis was performed. In brief, a score of 0 was given to sections showing no staining or membranous reactivity in <10 % of cancer cells, and 1+ was given to sections showing faint or barely perceptible membrane reactivity in >10 % of cancer cells; cells are reactive only in part of their membrane, 2+ was given to sections showing weak to moderate complete basolateral or lateral membranous reactivity in >10 % of cancer cells, and 3+ was given to specimens showing strong complete basolateral or lateral membranous reactivity in >10 % cancer cells. Scores of 0 and 1+ were considered negative for HER2 overexpression, whereas scores of 2+ and 3+ were considered to be HER2 overexpression. Although a FISH test is recommended in HER2+ cases, this could not be done due to economic constraints.

Statistical Analysis

The statistical analysis was done using MedCalc software version 12. Observed survivals were calculated with Kaplan–Meier curves. Categorical variables were compared with chi-square analysis. A p value of <0.05 was considered significant.

Results

There were 20 females and 50 males. The age ranged from 30 to 71 years (mean 52.97 ± 7.08 years; 95 %CI 51.2835–54.6594). The histology revealed 20 (28.6 %) intestinal type and 50 (71.4 %) diffuse gastric cancer. HER2 overexpression (2 + or 3 + immunostaining) on IHC was found in 15 (21.4 %) samples and no overexpression (0 or 1+ immunostaining) in the remaining 55 (78.6 %) samples (Table 1, Fig. 1). In all, only 20 patients had intestinal-type cancer and 9 (45 %) of these were HER2 positive compared to 6 (12 %) of 50 with diffuse-type cancer and this was statistically significant ($p = 0.006$). Interestingly, 56 (80 %) had poorly differentiated cancer (most signet ring type). No association was found between HER2 overexpression and age, gender, stage, and grade of tumor. Of the 45 patients who underwent radical gastrectomy with D2 lymph node dissection, two patients developed left supraclavicular lymph node metastasis in follow-ups at 18 and 24 months, respectively. Both these patients had signet ring gastric

cancer and were HER2 negative. Kaplan–Meier disease-free survival (DFS) curves for 11 HER2-positive and 34 HER2-negative patients with stage I–III gastric/GEJ cancer are shown in Fig. 2 ($p = 0.6$).

Discussion

This study was undertaken in a tertiary care referral hospital of northern India to assess the HER2 status in patients with gastric and GEJ cancer. Only one detailed report has been published in indexed literature from India [14] while the present paper was in review, showing a very high rate of HER2 expression (44.2 %) in gastric cancer. This high expression of HER2 has been questioned by a note from another Indian center that quotes its findings (unpublished data from hospital database) to approximately 15 % [15]. A lack of interest in pursuing HER2 status in advanced gastric cancer is possibly because trastuzumab is still out of reach of a large majority of Indian patients who do not have any kind of medical insurance to cover the cost of such treatment. Similar sentiment is shared by our neighboring country China, which estimated an annual increase in fiscal expenditures to be approximately \$1 billion rendering the treatment not cost-effective [16]. Nevertheless, this study brings out certain facts discussed as follows that need to be confirmed on a larger subset of patients in future studies.

The HER2 is overexpressed in a minority of patients with gastric and GEJ cancer ranging from 2 to 45 % [17]. Overexpression of HER2 protein in gastric cancer using IHC was first described in 1986 [18]. In a Japanese series, HER2 overexpression was found in 23 and 27 % by IHC and FISH, respectively, in 200 resected specimens [19] and 16 % in a Korean series [20]. In the ToGA clinical trial, an overall HER2 expression (IHC 3+ and/or FISH +) was reported as 22 % in 2,168 patients and a higher rate of HER2 positivity was found in GEJ than in gastric cancer samples (34 vs. 20 %) [12]. The HER2 overexpression in our study was 21.4 %. We included patients with IHC score 2+ and 3+. FISH analysis could not be carried out in HER2 2+ cases due to economic constraints, and hence, our HER2 positivity rate may actually be lower.

HER2 overexpression has been reported to be associated with advanced stage and poor overall survival in breast cancer. However, conflicting data are available for the same in gastric cancer. A Chinese study recently reported HER2 positivity to be an independent poor prognostic factor ($p = 0.042$) for recurrence in differentiated gastric cancers [21]. In another study involving 260 gastric cancer samples, HER2 expression was an independent prognostic factor, and the intensity of HER2 staining correlated with tumor size, serosal invasion, and lymph node metastases [22]. Nakajima et al. stated that even in early-stage tumors, HER2 overexpression is the second poorest prognostic variable after nodal status [23]. Park et

Table 1 HER2 expression in gastric or GEJ cancer in 50 patients

Criteria	HER2 positive (15)	HER2 negative (55)	p value
Age (years)			0.19
≤50	8	17	
>50	7	38	
Gender			0.89
Males	10	40	
Females	5	15	
Stage (TNM)			II + III vs. IV
I	0	0	0.6
II	2	10	
III	9	24	
IV	4	21	
HPE (Lauren's type)			0.006
Diffuse	6	44	
Intestinal	9	11	
Grade			WD + MD vs. PD
WD	0	3	0.72
MD	3	8	
PD	12	44	

MD moderately differentiated, PD poorly differentiated, TNM tumor–node–metastasis, WD well differentiated

Fig. 1 Immunoperoxidase (IP) staining for HER2 receptors. **a** Malignant cells show minimal membrane staining and are negative for HER2 (IP, $\times 400$). **b** Strong positive membrane staining of tumor cell membrane (IP, $\times 400$)

al. from Korea found age, tumor–node–metastasis (TNM) stage and amplification of HER2 to be independently related to survival by multivariate analysis in their study of 182 gastric cancer patients who underwent curative surgery. Intestinal-type cancers also exhibited higher rates of HER2 amplification than did diffuse-type cancers ($p < 0.05$) [20]. On the contrary, recent studies involving a large cohort of patients found no association of HER2 overexpression with patient survival or TNM stage [24, 25]. No association was found between HER2 overexpression and age, gender, stage, and grade of tumor in our study. The DFS was also not different in the HER2-positive vs. HER2-negative patients. However, this could be due to the short follow-up of just 2 years and a small subset of patients.

HER2 overexpression appears to be associated with intestinal-type gastric cancer [25], and therefore, subclassification of gastric adenocarcinomas as intestinal or diffuse type may have implications for therapy. The specific reasons for the overexpression of HER2 in intestinal-type gastric carcinomas are complex and demand further study. A

correlation has been drawn to breast cancer wherein HER2 gene amplification is a common feature in invasive ductal carcinomas and an uncommon feature in lobular carcinomas. In gastric cancer, HER2 gene amplification is reportedly inversely associated with E-cadherin mutations [26]; the latter are associated with diffuse gastric and lobular invasive breast cancers but rare in intestinal gastric and ductal invasive breast cancers [11]. We found that 80 % patients had poorly differentiated cancer. In all, only 20 patients had intestinal-type cancer and 9 (45 %) of these were HER2 positive compared to 6 (12 %) of 50 with diffuse-type cancer. Thus, HER2 expression was significantly more in intestinal-type cancer ($p = 0.006$) in our study.

It is imperative to diagnose the HER2-positive cases with accuracy because it opens a new channel of treatment with trastuzumab. It has been reported that HER2 expression is different in gastric and breast cancers. Gastric cancer shows considerable intratumoral heterogeneity, and positivity rates may differ in samples taken from resected specimen compared to those taken as a biopsy [24]. Thus, pathologists worldwide argue for a gastric cancer scoring system that is slightly different from the scoring system currently being used for breast cancer [27]. An understanding of the scoring modifications is required for proper stratification of gastric cancer patients for treatment. The ToGA trial used a modified four-tier scoring that was developed by Hofmann et al. [12, 28]. In a subsequent validation study, this scoring system was also found reproducible among different pathologists [29]. An interesting study [25] published very recently examined HER2 overexpression by IHC and HER2 gene amplification by FISH in 169 patients (99 gastric adenocarcinomas, 70 GEJ carcinomas) using scoring schemes proposed by both the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) and the those published by the ToGA trial. Overall, 19 adenocarcinomas were HER2 positive, defined as either a HER2/CEP17 ratio > 2.2 and/or a 3+ HER2 IHC score with either the ASCO/CAP or ToGA scoring schemes. Of the 19 HER2-positive adenocarcinomas, eight (42 %) exhibited a characteristic strongly intense basolateral membranous staining pattern that would be interpreted as negative (1+) using the accepted ASCO/CAP scoring scheme for HER2 assessment in breast carcinoma, but were correctly labeled as 3+ positive using the

Fig. 2 Kaplan–Meier DFS curves of HER2-positive vs. HER2-negative patients. Legend: DFS curves of 11 HER2-positive and 34 HER2-negative patients with stage I–III gastric/GEJ (Siewert type III) cancer showing no statistically significant difference (log rank test: $p = 0.6$)

proposed ToGA scoring scheme. Of the 19 HER2-positive adenocarcinomas, eight (42 %) demonstrated heterogeneous HER2 protein expression by IHC. The authors cautioned that a characteristic basolateral membranous pattern (often heterogeneous) of HER2 expression was observed in both gastric and GEJ adenocarcinomas rendering routine evaluation of HER2 status on small tissue samples challenging.

It is important that pathologists and diagnostic laboratories learn and use the modified criteria for assessing HER2 status in gastric/GEJ cancer because a large number of new specimens will need to be tested for HER2 status by both IHC and FISH in the days to come as trastuzumab has now been approved by the Food and Drug Administration for use in metastatic gastric and GEJ adenocarcinoma [30].

Conclusion

HER2 overexpression is found only in a minority of patients with gastric and GEJ cancers in the Indian population. It is more likely to be overexpressed in the intestinal-type adenocarcinoma and does not appear to be a prognostic marker. HER2 status assessment as a routine in the workup for metastatic gastric and GEJ will become important in the near future.

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