HER2 Expression in Gastric Adenocarcinoma—a Study in a Tertiary Care Centre in South India

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Abstract According to the literature available, HER2(human epidermal growth factor 2)status in gastric carcinoma has been studied worldwide, however there is a paucity of published data from India. Hence, this study was taken up to evaluate HER2 overexpression in gastric adenocarcinoma patients and to assess the relationship between its expression and clinicopathological tumor parameters. Prospective study was conducted in a teaching hospital over a period of 27 months. Total or subtotal gastrectomy resection specimens and small biopsies were included in the study. Immunohistochemistry (IHC) was carried out on all the cases to evaluate the expression of HER2 in formalin-fixed paraffin-embedded tissue samples. Fluorescence Insitu Hybridization (FISH) was done for equivocal cases on IHC. The data was analyzed using Chi square test / Fisher's Exact Test. Odds ratio was computed between HER2 and other pathologic variables. HER2 overexpression was confirmed in 16 (27.6 %) cases of which 15 (93.8 %) cases were of intestinal type whereas only 1 (6.2 %) case of diffuse type adenocarcinoma. Hence, HER2 positivity was significantly

more common in the intestinal type of gastric cancer compared to the diffuse type ($p=0.045$). Positivity of HER2 was more in small biopsies as compared to the resection specimens in this study but the p value was not significant. There was no difference in HER2 overexpression in relation to the age, gender, tumor site, tumor differentiation and stage. HER2 overexpression is more prevalent in the intestinal subtype. The relatively high percentage of HER2 positive tumors may provide a useful target for immunotherapy of these cancers.

Keywords HER2 · Gastric cancer · Grade · Immunohistochemistry · Lauren's classification

Introduction

Gastric cancer is one of the leading causes of cancer mortality in the world [1]. The annual incidence of gastric cancer in India is 10.6 per 100,000 population [2].

New targeted therapy for advanced gastric carcinoma depends on the evaluation of HER2 gene status. Currently, the clinical trials using anti HER2 antibodies like Trastuzumab for advanced gastric carcinoma and metastatic gastric carcinoma are underway (Trastuzumab for Gastric Cancer (ToGA) study) [3]. The TOGA trial has shown to improve the survival in patients with advanced HER2 positive gastric adenocarcinoma by combining the conventional chemotherapeutic agents with Trastuzumab.

There is literature available from various parts of the world regarding HER2 status in gastric carcinoma but there is a paucity of published data from India. This study was therefore undertaken to score HER2 overexpression in gastric adenocarcinoma and to study the correlation between HER2 positivity and clinicopathological factors.

Key Messages HER2 status is more common in the intestinal type of gastric adenocarcinoma. A higher rate of HER2 positivity is seen in small biopsies as compared to the resection specimens due to better antigen preservation in small biopsies as they have shorter cold ischaemic time as a result of quicker fixation.

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Subjects and Methods

Specimens from the patients with gastric adenocarcinoma diagnosed at a teaching hospital in South India from Jan 2012 to March 2014 were studied. Total or subtotal gastrectomy resection specimens and small biopsies with more than 5 bits were included in the study. None of the patients had received any previous treatment. Histopathological reporting of H&E stained slides was done and tumors were classified as intestinal and diffuse type according to Lauren's classification. Intestinal type of adenocarcinoma was considered as well differentiated with >95 % glands (Grade I), moderately differentiated (Fig. 1) with 50 to 95 % glandular pattern (Grade II) and poorly differentiated with a predominantly solid pattern and <50 % glands (Grade III). Immunohistochemistry (IHC) for HER2 was performed on formalin-fixed paraffin-embedded sections of specimens. Primary antibody against HER2 protein (Biogenex-CB11) was used with rabbit anti mouse secondary antibody. Positive and negative controls were run with each batch of slides. The immunostained slides were evaluated using criteria given by Hofmann et al. and modified by Davidson and Pai (Table 1) [4, 5]. Tumors with IHC score of 2+ were subjected to fluorescence in situ hybridization (FISH) assay. HER2 expression was considered positive if IHC 2+ was positive by FISH and negative if FISH was negative. HER2 status was then correlated with the clinicopathological factors like age, gender, tumor location, histological differentiation and pTNM classification.

Statistical Analysis

The data was analysed using Statistical Package for Social Sciences (SPSS, Ver 17). Chi square test / Fisher's Exact Test was used to test the association between HER2 expression with demographic and pathological variables. Odds ratio was computed between HER2 and other variables.

Results

A total of 58 specimens were studied with the age ranging from 30 to 80 years. The mean age being 58.8 years. Out of 58 patients, 43 were males and 15 were females. The most common symptoms of clinical presentation for gastric adenocarcinoma patients in our study was found to be vomiting (38 %) and dyspepsia (38 %). In our study, we found antrum (39.7 %) to be the most common site of gastric carcinoma followed by pylorus. Fundus and entire stomach (Linitis plastica) was found to be the least common. We evaluated HER2 overexpression in 58 cases, out of which 41 were small biopsies and 17 were surgical resection specimens. Histological typing was done for all cases according to Lauren's

classification. Forty three patients had intestinal type and 15 had diffuse type adenocarcinomas. Amongst 41 small biopsies, 29 were intestinal type and 12 were of diffuse histological type. Of a total of 17 resection specimens, 14 were of intestinal type and three cases were of diffuse morphology. Majority of the cases with intestinal type of gastric adenocarcinoma presented with grade II tumors (55.8 %). We received 17 resection specimens, out of which ten patients had (58.8 %) stage III disease. Hence, majority of patients who underwent surgery for gastric carcinoma presented in stage III. Gastric mucosa adjacent to the tumor was evaluated which showed various additional findings like gastritis, intestinal metaplasia and H.pylori infection. Intestinal metaplasia was seen in both small biopsies and resection specimens. Hence, it was the most common finding in our study as it was seen in 23 out of 58 (39.7 %) patients. H.pylori infection with associated gastritis was seen in 13 out of 17 (76.5 %) resection specimens.

Immunohistochemistry was carried out on 58 cases of gastric carcinoma and studied for HER2 overexpression. Out of 58 cases, 14 patients (24 %) showed overexpression (3+) (Fig. 2) and 40 (69 %) were negative (score 0 or 1+). Four cases (7 %) had a score of 2+ and were considered equivocal; However, FISH was carried out for these four equivocal cases and two cases turned out to be FISH positive. Hence, HER2 positivity was seen in 16 patients (27.6 %).

Of a total of 16 cases with overexpression of HER2, 15 (93.8 %) cases were of intestinal type whereas, only 1 (6.2 %) case was of diffuse type adenocarcinoma. The difference in the positivity rate for HER2 between the two histological groups of adenocarcinoma was found to be statistically significant ($p=0.045$). Out of these 15 cases of intestinal adenocarcinomas showing HER2 positivity, 12 were of grade II and 3 were of grade III. There were no positive cases in grade I. Hence, HER2 positivity is higher in moderately to poorly differentiated intestinal type of adenocarcinoma.

When HER2 positivity was analysed according to type of specimen, we observed a higher rate of HER2 positivity in small biopsies as compared to the resection specimens. Out of 41 small biopsies 14 (34.1 %) showed HER2 overexpression, whereas only two out of 17 (11.8 %) resected specimens showed HER2 positivity. HER2 positivity rates and its relation to various clinicopathological factors is summarized in Table 2.

HER2 immunostaining was heterogeneous in all positive cases. There was no difference between the HER2 overexpression cases and low expression (negative) cases in relation to age, gender, tumor site and tumor differentiation.

Discussion

Carcinoma stomach is one of the most common malignancies worldwide. Surgical resection is the mainstay of treatment for

Fig. 1 Microphotograph of moderately differentiated type of gastric adenocarcinoma-Intestinal type (H&EX400)

patients with early-stage gastric cancer. However, most patients are diagnosed when the tumor is at an unresectable stage and for these patients, systemic chemotherapy is the main treatment option [6]. The survival rate of these patients with advanced disease remains poor with perioperative chemotherapy or adjuvant chemoradiation [7]. The current therapeutic options for patients with gastric carcinoma appear to have reached a plateau of effectiveness and new treatment modalities including targeting agents need to be considered.

Various prognostic factors for determining the survival for gastric carcinomas include age, tumor stage, location, tumor size and histological type. Stage is the most important prognostic factor for gastric carcinoma followed by histologic type. However, patients in the same stage and histological type have varied prognosis, therefore additional parameters need to be identified in addition to stage and tumor type in order to identify the biological subsets of this disease.

Carcinogenesis in the stomach, like that in the other organs, is a multistep process involving the accumulation of several genetic alterations, which include the activations of oncogenes and the inactivation of tumor suppressor genes. Several genetic alterations associated with gastric carcinomas showing HER2

overexpression at the DNA or protein levels may form a specialized subgroup in terms of their clinicopathologic characteristics and optimal therapeutic approaches. HER2/NEU is a gene which regulates the production of human epidermal growth factor 2 (HER2) protein. HER2 protein is a growth factor of the EGFR family with intrinsic protein tyrosine kinase activity and its increased activity is an assumed mechanism of cell transformation. Activation of EGFR triggers a network of signalling processes that promote tumor cell proliferation, migration, adhesion and angiogenesis and decrease in apoptosis [8]. Amplification of HER2 gene has been demonstrated in adenocarcinomas in different sites like breast, stomach, colon and over expression is associated with poor prognosis [9–13]. In 2010, the “Trastuzumab for Gastric Cancer (ToGA)” study, a randomized, multicenter, international, phase 3, controlled trial was designed to assess the clinical efficacy and safety of the anti-HER2 agent trastuzumab as an addition to chemotherapy for first-line treatment of advanced or metastatic gastric cancer overexpressing HER2.

Gastric cancer exhibits unique HER2 immunostaining characteristics compared to breast cancer, including high incidence of tumor heterogeneity (defined as 30 % of tumor cells

Table 1 HER2 Immunohistochemistry scoring for gastric adenocarcinoma in biopsy and resection specimens

Type of specimen	Reactivity characteristics	Score / classification
Resection specimens	No reactivity (at X 40 magnification)	0/negative
	Faint/barely perceptible partial membranous reactivity in >10 % of tumor cells (at X 40 magnification)	1+/negative
	Weak/moderate complete OR basolateral/lateral membranous reactivity in >10 % of tumor cells (at 10 X magnification)	2+/equivocal
	Moderate/strong complete OR basolateral/ lateral membranous reactivity in >10 % of tumor cells Unequivocal membranous staining at low magnification (at 4X magnification)	3+/positive
Small biopsy specimens	Any single cluster of tumor cells (>=5 cells) demonstrating IHC 3+ staining characteristics 3+/positive	3+/positive

Fig. 2 HER2 positive(3+ score) immunostained microphotograph of moderately differentiated adenocarcinoma in a small biopsy (X400)

staining positive or only focal staining of tumor cells in up to 30 % of HER2 positive cases) [4, 14]. Another key difference from breast cancer staining is that HER2 positive gastric carcinomas are usually of the gland-forming intestinal type and may show incomplete, basolateral, or lateral staining in addition to complete membrane staining and all these are considered as positive with immunohistochemistry. These

Table 2 Relationship between HER2 expression and clinicopathologic features in patients with gastric cancer

	Number	Positive	Percentage
Sex			
Male	43	12	27.9
Female	15	4	26.7
Age			
>65 years	22	7	31.8
<65 years	36	9	25.0
Type of specimen			
Small biopsies	41	14	34.1
Resection	17	02	11.8
Histological type			
Intestinal	43	15	34.9
Diffuse	15	1	6.7
Differentiation			
Well-Diff	6	0	0
Mod-Diff	24	12	50.0
Poorly-Diff	13	3	23.1
Stage of cancer (n=17)			
Stage I	1	0	0
Stage II	6	0	0
Stage III	10	2	11.6
Stage IV	0	0	0

differences have been taken into account when devising the optimal HER2 testing protocol for gastric cancer by Hofmann et al. [4]. Their study suggested that this scoring system shows a high level of concordance of 93.5 % between IHC and FISH methodologies to detect HER2 positivity in gastric cancer.

Recent studies have shown the significance of HER2 overexpression in the prognosis and therapy of gastric carcinoma. It has been approved for the treatment of metastatic HER2 positive gastric adenocarcinomas. Hence, HER2 status should now be routinely included in the diagnostic work up of the patients with advanced or metastatic gastric cancer.

The present study provides information about the clinical features, histological type and overexpression of HER2 by immunohistochemistry in a tertiary care centre over a period of 27 months.

Gastric adenocarcinomas have been reported to show a wide range of over expression of HER2 protein ranging from 10.7 to 44.2 % [2, 4, 15–21]. It is clearly evident from Table 3 that there is a wide variation with reference to overexpression of HER2 in various ethnic groups worldwide. This may be attributed to various factors such as methodology adopted, standardization of the procedure, differences in the reporting protocol, tumor heterogeneity and variations of HER2 positivity in the populations studied.

Our observed rate of HER2 protein over expression (27.6 %) correlates with the range previously reported as in Table 3. However, Sekaran et al. conducted a study in Hyderabad and got a very high HER2 positivity of 44.2 % [2]. Another study in India performed by Lakshmi V et al. reported HER2 positivity of 35.9 % [19].

As in breast carcinoma, where HER2 gene amplification is common in invasive ductal carcinoma and an uncommon

Table 3 Comparison of rate of HER2 positivity in various studies

Authors	Year of study	Population studied	Number of patients studied	Percentage of HER2 positivity
Hofmann et al. [4]	2007	Germans, Chinese and Mexicans	178	10.7 %
Raziee et al. [15]	2007	Iranians	100	26 %
Marx et al. [16]	2009	Germans	166	19 %
Xie et al. [17]	2009	Chinese	218	18.8 %
Lee et al. [18]	2010	Australians	178	17.4 %
Sekaran et al. [2]	2011	Indians	52	44.2 %
Lakshmi V et al. [19]	2014	Indians	78	35.9 %
Ogun et al. [20]	2014	Nigerians	36	11 %
Yoshida et al. [21]	2014	Japanese	207	17 %
Present study	2015	Indians	58	27.6 %

feature in lobular carcinoma, the intestinal type of gastric cancer shows higher prevalence of HER2 amplification in contrast to the diffuse type.

In this study, HER2 positivity was found in 16 cases, out of which 15 (93.8 %) were of intestinal type and only 1 (6.2 %) was of diffuse type. The Chi square test revealed a *P* value of 0.045 which was statistically significant. This finding was consistent with many other studies. HER2 positivity was more common in the intestinal type than diffuse types in various studies as shown in Table 4. This difference in HER2 positivity in different histologic type supports the idea of substantial molecular differences between the histologic tumor types, which may develop through different molecular alterations. For example, reduced or abnormal E-Cadherin expression is a molecular feature that is strongly linked to the diffuse type of gastric carcinoma. However, study conducted by Sekaran et al. showed similar HER2 positivity in both intestinal and diffuse types of gastric carcinomas [2].

Out of a total of 41 small biopsies taken for our study, 14 (34.1 %) cases were HER2 positive whereas, in 17 resected specimens, only two cases (11.8 %) were HER2 positive. Hence, there is a much higher rate of positivity observed in biopsy specimens compared to the resected specimens but the calculated *P* value is not significant. Such findings have also

been described by Lee et al. and Jeung et al. [18, 24]. Davidson and Pai have suggested that a higher rate of positivity in biopsies is due to better antigen preservation in biopsies as they have shorter cold ischaemic time as a result of quicker fixation [5]. Cold ischemic time is the time between tissue removal and the initiation of formalin permeation of the tissue and is an important pre-analytic variable. The progressive loss of activity of labile macromolecules after the surgical interruption of blood flow leading to tissue ischemia, acidosis, and enzymatic degradation has been documented [25–29]. Aktas et al. have studied the effect of cold ischaemic time on HER2 positivity in breast resection specimens [30]. They have suggested that it is best to keep cold ischemic time as minimal as possible and recommendation of <1 h is a prudent guideline to follow. However, following this guideline will require changes in practice in many institutions and will require a team approach. The surgeons will be required to submit resection specimens promptly to pathology laboratories. To avoid delay in sectioning, pathology laboratories should designate a pathology assistant, for promptly processing large specimens, who would section the tumor immediately and then allow it for overnight fixation. Therefore, according to this study, it is suggested that one should prefer to do immunohistochemistry on small biopsies

Table 4 Comparison of HER2 positivity in relation to the histological type

Authors	Year of study	Percentage of HER2 positivity according to the histological type	Diffuse type
		Intestinal type	
Hofmann et al. [4]	2007	83.3 %	16.7 %
Raziee et al. [14]	2007	74 %	26 %
Kim et al. [22]	2007	67.9 %	32.1 %
Marx et al. [15]	2009	92 %	8 %
Tafé et al. [23]	2011	95 %	5 %
Lakshmi V et al. [19]	2014	84.6 %	15.4 %
Present study	2015	93.8 %	6.2 %

because of its greater sensitivity to HER2 in cases where chemotherapy with Trastuzumab is planned. However, Yoshida et al. have found similar positivity in both the resection and biopsy specimens using IHC and FISH in their study [21].

There is a higher rate of HER2 positivity in moderately and poorly differentiated types of intestinal adenocarcinoma which is in concordance with the study conducted by Kim et al. in which 51.8 % of HER2 positive cases were moderately differentiated type of intestinal adenocarcinoma [22]. Similarly, Marx et al. found that 60 % of HER2 positive cases were moderately differentiated. Well differentiated tumors in most of the studies had a very low rate of HER2 positivity [16]. This explains the bad prognosis associated with HER2 positivity [31, 32].

As gastric cancer is associated with poor outcome, the importance of research on novel prognostic and therapeutic markers cannot but be over emphasised. As discussed above, the importance of cold ischaemic time needs to be further analysed in gastric resection specimens in future studies. Increasing the sample size in future studies and designing prospective studies with close observation of survival, the utility of HER2 over expression as an important prognostic marker can be enhanced.

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