

Immuno-Histochemical Assessment of HER2NEU Expression in Gastric Adenocarcinoma in North Karnataka, India

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Abstract

Background and Objectives: Gastric cancer is the fourth most common cancer worldwide and ranks fifth in India. Surgical resection is curative in early stage gastric cancers. Most of the gastric cancers are diagnosed at an advanced stage necessitating multimodality treatment strategies. Based on the ToGA trial, the international regulatory agencies have recently approved trastuzumab in locally advanced and metastatic gastric and gastroesophageal adenocarcinomas expressing HER2. Since there are limited studies from India and no published data available from this part of North Karnataka, we undertook this study to evaluate the frequency of expression of HER2 in gastric and gastroesophageal adenocarcinomas and to correlate it with various clinicopathological variables. **Methodology:** The study was conducted in the Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka from May 2012 to January 2016. The samples included both endoscopic biopsies and gastrectomies. Histopathological slides from 70 cases were reviewed. Immunohistochemical staining for HER2 was performed in all the cases and Hoffman's gastric cancer scoring system was employed. The results of HER2 expression was correlated with various clinicopathological parameters. **Results:** HER2 positivity was seen in 16/70 cases (23%). 6 cases (8.5%) were equivocal and 48/70 cases (68.5%) were HER2 negative. HER2 positivity was more common in GEJ cancers and intestinal type of adenocarcinoma. However, it did not correlate with age, gender, grade and stage. **Conclusion:** HER2 positivity was noted in 23% of the cases. 23.4% of intestinal type and 21.7% of diffuse type were HER2 positive. HER2 positivity did not significantly depend on age, gender, tumour type, grade and stage. Hence, HER2 remains as an independent biomarker and should be tested in all patients of gastric cancer regardless of the clinicopathological findings for offering a personalized treatment.

Keywords: HER2- Gastric cancer- IHC

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Introduction

Gastric cancer accounts for the fourth commonest cancer worldwide. It is also second most common cause of cancer related deaths not only in the world but also in India (Almasi et al., 2015). Surgery is the curative procedure for the early gastric cancers but most of the patients are diagnosed at an advanced stage when the tumor is unresectable (Jung et al., 2013). The recent guidelines published by Japanese Gastric Cancer Association (JGCA) recommend the multidisciplinary approach to treat gastric cancer patients which advocates one or more of five options such as surgery (extended/palliative), chemotherapy, radiation therapy or palliative care (Baba et al., 2013).

In this regard, intense research is going on throughout the world towards new molecular targets such as HER2. Recently, HER2 gene amplification and protein expression has been recognized in many gastric cancers (Sekaran et al., 2012; Jung et al., 2013). These subset of patients

with gastric tumours expressing HER2 are the ones who respond to targeted therapy using trastuzumab (Ruschoff et al., 2012). Several studies are being undertaken worldwide regarding the expression of HER2 in gastric adenocarcinoma. However, there is paucity of such studies from India and especially from the region of North Karnataka as very few studies are reported so far. In view of the above, the present study was undertaken to study the frequency of HER2 expression in gastric and gastro-esophageal carcinomas and to correlate the HER2 positivity with pathological variables such as tumour type, grade and stage wherever possible.

Materials and Methods

The present study has been carried out in the Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka. This study comprised of both retrospective and prospective cases which included gastric and gastro-oesophageal

adenocarcinomas diagnosed both on small endoscopic biopsies and gastrectomy specimens during the study period from May 2012 to January 2016. The sample size including endoscopic biopsies and gastrectomy specimens were 70 cases.

Immunohistochemical assessment of HER2 was carried out in all the cases and further correlated with various clinicopathological variables like age, gender, tumour type and grade in all the cases. Tumour stage was correlated only in resection specimens. The H and E slides were assessed for the pattern of arrangement of tumour cells. The tumours were classified into intestinal and diffuse type based on the Lauren's classification. All the tumours were graded into well, moderate and poorly differentiated based on the composition of glands.

Sections from tumour proper without necrosis and haemorrhage were selected for immunohistochemical staining. IHC was performed on the respective tumour blocks cut and taken on the APES (aminopropyltriethoxysilane) coated slides. HER2 antibody was obtained from the Biogenex Company and the staining procedure was followed as per the protocol given by the company. A control was set up for HER2 staining for every batch of slides. HER2 positive breast cancer served as the control. After the HER2 immunohistochemical staining was complete, the data was tabulated separately as HER2 positive cases, equivocal cases and HER2 negative cases. Clinicopathological data such as age, sex, anatomic location of the tumour, histologic type, grade and stage of the tumour in gastrectomy specimens were separately tabulated. The relationship between various clinicopathological parameters was then correlated with HER2 positivity and p value was calculated. The data of different variables were analysed for the test of independence using appropriate chi-square test which has assumption of independence of tested variables as null hypothesis if $p > 0.05$ and existence of association or relation among the variables as alternative hypothesis if $p < 0.05$. The p value < 0.05 was considered for significance. HER2 was expressed in percentage with 95% confidence

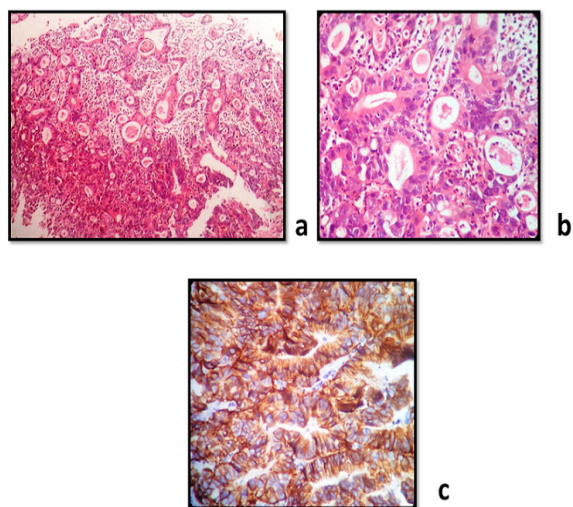


Figure 1. (a and b): Lauren's Intestinal subtype of adenocarcinoma (H&E, x100 and x400), (c): HER2 IHC showing strong complete/basolateral membranous staining in $>10\%$ tumour cells - 3+ Positive (x400).

interval. To describe the quantitative data such as age and gender, descriptive statistics such as mean and standard deviation were used.

Results

In the present study, 70 cases of gastric and gastro-esophageal carcinomas were analyzed for the expression of HER2 by IHC. HER2 positivity was found in 23% of the cases (16 out of 70 cases).

The HER2 expression observed in the present study has been correlated here-under with reference to age, gender, Lauren's histological type, grade and stage wherever possible.

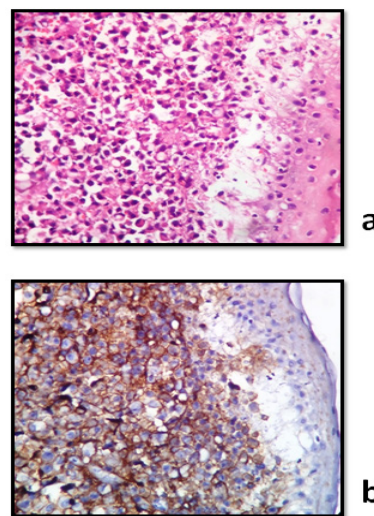


Figure 2. (a): GEJ tumour showing Lauren's Diffuse subtype of adenocarcinoma (H&E,x400), (b):HER2 IHC showing moderate to strong complete or basolateral membranous staining in $>10\%$ of tumour cells- 3+ Positive (x400).

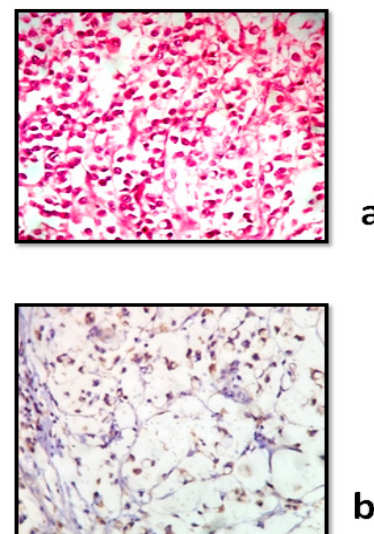


Figure 3. (a): Lauren's Diffuse subtype of adenocarcinoma (H&E, x400), (b) :HER2 IHC showing moderate to strong complete /basolateral membranous staining in $>10\%$ of tumour cells - 3+ Positive (x400).

Table 1. Correlation of HER2 Expression with Various Clinicopathological Factors

Clinicopathological factors	HER2 Positive (%)	HER Negative (%)	Equivocal (%)	p Value
Age in Years				
≤50 (26)	7 (26.9%)	18 (69.2%)	1 (3.8%)	0.448
>50 (44)	9 (20.4%)	30 (68.1%)	5 (11.3%)	
Gender				
Males (56)	14 (25%)	37 (66.07%)	5 (8.9%)	0.650
Females (14)	2 (14.2%)	11 (78.5%)	1 (7.14%)	
Location				
GEJ (15)	4 (26.6%)	9 (60%)	2 (13.3%)	0.616
Proximal (13)	2 (15.3%)	11 (84.61%)	0	
Distal (42)	10 (23.8%)	28 (66.6%)	4 (9.5%)	
Lauren's Classification				
Intestinal (47)	11 (23.4%)	33 (70.2%)	3 (6.3%)	0.995
Diffuse (23)	5 (21.7%)	15 (65.2%)	3 (13.0%)	
Differentiation				
Well (34)	9 (26.4%)	23 (67.6%)	2 (5.8%)	0.645
Moderate(10)	1 (10%)	8 (80%)	1 (10%)	
Poor(26)	6 (23%)	17 (65.3%)	3 (11.5%)	
Stage (n=16)				
I (3)	0	3 (100%)	0	>0.05
II (2)	0	2 (100%)	0	
III (9)	2 (22.2%)	7 (77.7%)	0	
IV (2)	0	2 (100%)	0	
Lympho vascular Invasion and Perineural Invasion (16)				
Present (14)	2 (14.2%)	12 (85.7%)	0	>0.05
Absent (2)	0	2 (100%)	0	

Discussion

In the present study, 70 cases of gastric and gastro-esophageal carcinomas were analysed for the expression of HER2 by IHC. HER2 positivity was found in 23% of the cases which was in line with the results of several other studies (9.4% to 44.2%) (Sekaran et al., 2012; He et al., 2013). The mean of HER2 positivity reported from eleven different studies from the samples

of patients across the globe was found to be 17.29 %. (Figure 1: Intestinal type adenocarcinoma : 3+ Positive). This wide variation of HER2 positivity in studies from different part of the world is contributed by many factors such as heterogenic aberrations in tumours from different geographic location, lack of uniform scoring criteria followed by pathologists for HER2 expression in gastric carcinoma across the world and the sample size (Kunz et al., 2012; Hoffmann et al., 2008). In the present study we

Table 2. HER2 Positivity Reported from Several Studies Across the World

Sl. No	Authors	Year	Geographic Location	Sample Size	Her2Positivity (%)
1.	Kunz PL et al., 2012	2012	California	169	22%
2.	Sekaran A et al., 2012	2012	India	52	44.2%
3.	Katoaka Y et al., 2013	2013	Japan	213	11.7%
4.	Shan L et al.,2013	2013	China	1463	9.8%
5.	He C et al., 2013	2013	China	197	9.64%
6.	Sheng WQ et al., 2013	2013	China	726	13%
7.	De carli DM et al., 2015	2015	Brazil	48	14.6%
8.	Panda S K et al., 2015	2015	India	150	18.7%
9.	Rajgopal I et al., 2015	2015	India	60	26.7%
10.	Laboissire RS et al., 2015	2015	Brazil	120	10.5%
	Present study	2016	India	70	23%

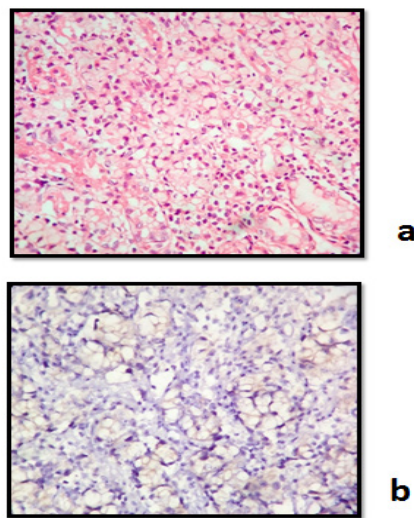


Figure 4. (a) Lauren's Diffuse Subtype of Adenocarcinoma (H&E, x400); (b), HER2 IHC showing weak to moderate complete or basolateral membranous staining more than 10% tumour cells - 2 + Equivocal (x 400)

have followed the scoring criteria given by Hoffman et al., (2008) in the TOGA trial, which is the most reliable scoring system used for gastric cancers.

In our study, we also noted extensive nonspecific cytoplasmic background staining in normal gastric mucosal cells in few cases. This non specific staining is a potential pitfall and should not be considered for scoring of HER2 expression. Similar findings were noted by Laboissiere et al who has attributed it to intrinsic clonal characteristics rather than a technical problem (Laboissiere et al., 2015). We also observed heterogeneous staining pattern with tumour cells showing both 2+ and 3+ areas in the same tumour. Similar observation was reported by Hoffman et al., (2008).

HER2 positivity in our study was 26.9% in patients less than 50 years of age and 20.4% in patients of more than 50 years of age. This showed that HER2 expression was independent of the age (p value = 0.448). Sekaran et al., (2012) found that HER2 expression was 47% in ≤ 50 years of age and 42% in patients > 50 years of age, thereby HER2 was found independent of the patients age. Similar studies also found that HER2 positivity was independent of age group (He et al., 2013; Panda et al., 2015; Laboissiere et al., 2015). However, Kataoka et al., (2013) observed that HER2 positivity was more frequent in the older age group (p value = 0.039) whereas Tewari et al., (2015) found that HER2 overexpression was more frequent in younger age group.

HER2 positivity was more commonly found in males with 25% as compared to females with a positivity rate of 14.24%. HER2 expression was found to be independent of gender (p value= 0.650). Similar findings were reported by few studies (Sekaran et al., 2012; Tewari et al., 2015; He et al., 2013; Panda et al., 2015; Laboissiere et al., 2015). However, HER2 expression was more frequently found in men with statistically significant correlation in the study conducted by Kataoka et al., (2013).

The most common location that we noted was in the distal stomach with the percentage being 60% followed

by 21.4% in the gastro-esophageal junction (GEJ) and the least in the proximal stomach accounting to 18.5%. In respect of association, HER2 expressivity was found to be independent of location of tumors ($p > 0.05$) although correlated positively with no significance. This was evidenced by the fact that 26.6% of the GEJ, 15.3% of the proximal stomach and 23.8% of the distal stomach were found to be HER2 positive. The maximum HER2 positivity was seen with the GE junction tumours. (Figure 2: GEJ tumour showing 3+ positive) In the ToGA screening program, which was the largest single study involving 3807 patients, Her2 positivity rate was 33.2% for GEJ tumours as compared to 20.9% for the tumours located in the stomach which was statistically significant (Jorgensen., 2014). Some of the previous researchers have found the location of the tumour independent of HER2 positivity. Kunz et al., (2012) reported 41.2% of the GEJ cancers and 58.5% of the gastric tumours with HER2 positivity in 10% of the GEJ tumours and 12.1 % of the Gastric tumours. Similarly, Sekaran et al., (2012) found that majority of the tumours were distally located and HER2 positivity was 40% in the proximal stomach cancers and 45% in distal cancers. De Carli et al., (2015) found that 88% of cases were distal cancers. HER2 positivity was 7% in gastric cancers and 14.6% in GE junction cancers. These results were found to be statistically significant ($p < 0.05$) with HER2 positivity more commonly found in GE junction cancers (Table 1).

In the present study, the majority of the tumours were of intestinal subtype. The HER2 positivity was seen in 23.4% of the intestinal type and 21.7% of the diffuse type (Figure 1 and Figure 3). However, it was found that HER2 positivity was independent of tumour histologic subtype with a $p > 0.05$ (0.995) which was also found by Sekaran et al., (2012). Kunz et al., (2012) found that HER2 positivity was associated with the intestinal type (19%) and diffuse type being 6%. HER2 positivity was more commonly encountered in 22.3% of the intestinal subtype and less commonly with mixed 15.4% (Kataoka et al., 2013). Similarly, Shan et al., (2013) found HER2 positivity in 44.4% of the intestinal type, 38.6% of diffuse subtype and 17% of mixed type. HER2 positivity correlated with the intestinal subtype in 16.8% cases, 2.3% with diffuse and 8.4% with mixed type.

Majority of the studies showed a positive correlation of HER2 positivity with the intestinal subtype. The very famous and revolutionary TOGA trial also showed a positive correlation with the intestinal subtype (34%) as compared to diffuse (6%) and mixed (20%) (Hoffmann et al., 2008).

In contrast with the data published by other authors the lower percentage of HER2 positive cases in our study in association with intestinal subtype could be partially explained by the increased frequency of diffuse type cancers in this part of North Karnataka. This could also account for the lack of statistically significant association between HER2 status and tumour type in our study.

The findings of the present study were in line with the study conducted by Sekaran et al., (2012). The probable causes of statistical non correlation with the intestinal subtype in our study include smaller sample size,

increased number of diffuse cases and the non availability of FISH for further confirmation of 2+/equivocal cases (6 cases). (Figure 4: Lauren's diffuse 2+ equivocal)

In GEJ tumours which accounted to 21.4% of the total cases, it was found that 73.3% were intestinal and 26.6% were diffuse. Thus HER2 positivity was more commonly associated with intestinal type of GEJ tumours which was also observed in the study conducted by Kunz et al., (2012).

In the present study there were 48.5% of well differentiated tumours, 14.2% were moderately differentiated and 37.14% were poorly differentiated. Of these, 26.4% of the well differentiated tumours were HER2 positive. Poorly differentiated tumours formed the next bulk of 23% whereas 10% of moderately differentiated tumours were HER2 positive. HER2 positivity was independent of tumour differentiation with a p value >0.05 which was also observed in several other studies (Sekaran et al., 2012; Tewari et al., 2015).

The majority of the tumours in our study were Stage III accounting for 56.25% of the cases followed by stage I in 18.5%. Of these, 25% of the cases in stage III were HER2 positive and 75% were HER2 negative. All the cases in stage I, II and IV were HER2 negative. In the present study, HER2 expression was found independent of stage of the tumour (p > 0.05) which was also observed in several studies (Kataoka et al., 201; Shan et al., 2013; He et al., 2013).

Lymphovascular invasion and perineural invasion were present in 87.5% of the gastrectomy cases. Of these, 14.25 % cases showed HER2 positivity. However, there was no significant correlation between HER2 positivity and lympho-vascular/perineural invasion (p value= 0.567). Similar observations were also made by Kataoka et al., (2013) and Laboissiere et al., (2015) who also found HER2 positivity to be independent of invasion (p>0.05).

In conclusion, HER2 expression was found in a significant number of patients with gastric and GEJ cancers in our study. It was more commonly expressed in males with a strong preference for GEJ location, Lauren's intestinal subtype and well differentiated tumours. Overall HER2 positivity was noted in 23% of the cases. In our study, 23.4% of intestinal type and 21.7% of diffuse type were HER2 positive and HER2 positivity did not significantly depend on age, gender, tumour type, grade and stage. Hence HER2 remains as an independent biomarker and should be tested in all patients of gastric cancer regardless of the clinicopathological findings for offering a personalized treatment.

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