



## Original Research Article

## HER2/neu expression in gastric carcinoma and its association with Helicobacter pylori infection and other clinicopathological parameters

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## ABSTRACT

**Background:** Gastric cancer is the 5<sup>th</sup> most common malignancy and 3<sup>rd</sup> leading cause of cancer death worldwide. Most cases are diagnosed in advanced stages making treatment difficult. International regulatory agencies have recently approved trastuzumab therapy in locally advanced and metastatic gastric adenocarcinomas expressing HER2.

**Aims:** To find HER2/neu expression in adenocarcinoma of stomach and correlate with clinicopathological features and study H.pylori positivity in HER2 positive cases.

**Settings and Design:** The ambispective, observational study was conducted in the department of Pathology (July 2017 to June 2019).

**Materials and Methods:** Seventy eight cases of gastric adenocarcinoma were studied, both endoscopy guided biopsy or gastrectomy. Expression of HER2/neu and detection of Helicobacter pylori was done using immunohistochemistry. HER2 expression was correlated with clinicopathological parameters and H.pylori infection.

**Statistical Analysis used:** Fisher's exact test, chi square test and p value <0.05 was considered significant.

**Results:** HER2/neu was positive in 26.92% of cases, 16.67% were equivocal and 56.41% were negative. 55.13% of intestinal type were HER2 positive. Grade 1 tumor showed more HER2 positivity (42.31%). HER2 positivity was independent of other parameters like age, sex and location of tumor. H.pylori was positive in 24.36% of HER2/neu positive cases.

**Conclusions:** HER2 positivity correlated with Lauren's intestinal type and grade 1 of tumor. HER2 is an independent biomarker regardless of other clinicopathological features. Though few Her2 positive cases showed H. pylori positivity, more larger studies are required to establish statistically significant association between HER2 positivity and H.pylori infection.

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### 1. Introduction

Cancer is considered as one of the most dreadful maladies and still stands as formidable challenge to the ingenuity of human mind to decipher its cause with precision and thereby find an effective way of treatment. Gastric carcinoma is the 5<sup>th</sup> most common cancer and third leading cause of cancer related death in the world.<sup>1</sup> In India it ranks as the 5<sup>th</sup> most common malignancy in males and 7<sup>th</sup> most common in females. Gastric carcinoma is the second leading cause of death in both sexes.<sup>2</sup> Scientists in their persistent effort

to probe into the cause of cancer have made significant inroads into the phenomena and are on the road to success to decipher its cause and find a cure. Continuous efforts are put to identify certain specific biological markers that could help in diagnosing the disease early and also help in improving the targeted therapy. Markers like HER2, E-cadherin, EGFR etc. are currently used to evaluate prognosis of disease.<sup>3,4</sup>

HER2 is a proto-oncogene, located in chromosome 17q21. It encodes a transmembrane protein having tyrosine kinase activity. It is also known as ERBB2 and CerBB2. It regulates signal transduction pathway causing cell growth and differentiation.<sup>5</sup> It is a member of epidermal growth

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factor receptor family. Accurate evaluation of expression of HER2 protein can be a help to identify eligible candidates for new targeted therapy.<sup>6</sup>

As we all know, prevention is better than cure, so we can use some preventive measures or some therapies to prevent and cure gastric carcinoma. There are several causative agents of gastric carcinoma like *H. pylori*, salt and food preserved with salt, obesity, smoking, alcohol, serum cholesterol level and HbA1c level.<sup>7</sup> Among all the causes, *H. pylori* is the most important causal factor. Stomach was thought to be a virtual desert for microbes because of its high acidity. However, among all species of bacteria, *H. pylori* lives in stomach. It was the first bacteria observed to behave as carcinogen. *H. pylori* can initiate precancerous lesion like atrophic gastritis and intestinal metaplasia. Further progression to dysplasia and carcinoma no longer require the presence of *H. pylori*.<sup>8</sup>

## 2. Aims and Objectives

1. To study HER2/neu expression in cases of adenocarcinoma of stomach
2. Correlating HER2/neu expression with clinicopathological features like-
  - a) Age
  - b) Sex
  - c) Location of tumor
  - d) Histopathological type
  - e) Histopathological grade
3. To find out frequency of *H. pylori* infection in adenocarcinoma of stomach by IHC.
4. To find out frequency of *H. pylori* positivity in gastric cancer patients showing HER2 expression.

## 3. Materials and Methods

The present study was conducted in the department of Pathology for a period of two years (from July 2017 to June 2019). Approval from the Institutional Ethics Committee was taken for the study.

### 3.1. Inclusion criteria

Histologically diagnosed cases of adenocarcinoma by routine H&E of subtotal and partial gastrectomy specimen and endoscopic biopsy from stomach were included in the study.

### 3.2. Exclusion criteria

Patients who had received chemotherapy.

There were 78 cases of gastric adenocarcinoma out of which, 58 were endoscopy guided biopsies and 20 were gastrectomies. Specimens were routinely processed and fixed overnight in 10% buffered formalin. Grossing of the specimens were done as per the AJCC guidelines. Four

to five micrometer thick formalin fixed, paraffin embedded tumor sections were stained with Haematoxylin and Eosin stain. Histological typing was done according to Lauren classification & histologic grading was done according to World Health Organization classification.

In our study, we have classified gastric cancer according to Lauren classification.

### 3.3. Intestinal type 2 Diffuse type

Microscopic grades as per WHO guidelines:

Gx - Grade cannot be assessed.

G1 - well differentiated adenocarcinoma - >95% gland formation in tumor tissue.

G2 - Moderately differentiated adenocarcinoma - 50-95% gland formation in tumor tissue.

G3 - Poorly differentiated adenocarcinoma - <50% gland formation in tumor tissues.

The sections were then stained with HER2 and *H. pylori* antibody separately and were studied. Immunohistochemistry scoring for HER2 expression was done for biopsy and gastrectomy specimens as given in table 1.<sup>9</sup>

## 4. Results

Total number of 78 cases of gastric adenocarcinoma were studied. The age range of patients was from 22 to 87 years with a mean age of  $60.08 \pm 13.55$  years. Maximum number of patients belonged to the age group 70-79 years (25.64%). Out of 78 cases, 53 (67.95%) were males and 25 (32.05%) were females. There was a male predominance with a male to female ratio of 2.12:1. According to Lauren's classification, 55.13% of cases were of intestinal type of gastric adenocarcinoma. HER2 positivity was seen in 26.92% (21 cases), 13 cases (16.67%) were equivocal and 44 cases (56.41%) were negative (Figure 1). HER2 did not show significant correlation with age and sex of patients (Table 2).

The commonest location of gastric adenocarcinoma in the present study was distal stomach (73.08%) followed by proximal stomach (19.23%) and gastroesophageal junction (7.69%). HER2 positivity was more in tumor located in proximal stomach (46.67%). Tumor located in distal stomach showed least percentage of HER2 positivity (21.05%) (Table 2). There was a significant correlation ( $p$  value = 0.01) of HER2 with Lauren's intestinal type (Table 3). Maximum no. of cases showing HER2 positivity belonged to grade I (42.31%). There was a statistically significant correlation between tumor grade and HER2 positivity (Table 4). There was no significant correlation of HER2 expression with *H. pylori* positivity (Tables 5 and 6).

Out of the 78 subjects included in our study, 20 cases were gastrectomies. Separate statistical analysis was performed to find out the correlation of HER2 positivity

**Table 1:** IHC Scoring criteria of HER2/neu overexpression in gastric adenocarcinoma<sup>9</sup>

Score	Surgical Specimen	Biopsy	HER2 overexpression assessment
0	No membranous staining or staining of <10% of the tumor cells	No membranous staining or staining only in rare cells (less than 5 cohesive cells)	Negative
1+	Staining I weak or detected in only one part of the membrane in $\geq$ 10% of the cells	Staining is weak or detected in only one part of the membrane of at least 5 cohesive cells	Negative
2+	Moderate/weak complete or basolateral membranous staining in $\geq$ 10% of the cells	Moderate/weak complete or basolateral membranous staining of at least 5 cohesive cells	Equivocal
3+	Strong complete or basolateral membranous staining in $\geq$ 10% of the neoplastic cells	Strong complete or basolateral membranous staining of at least 5 cohesive cells	Positive

**Table 2:** Correlation between age, sex & location of adenocarcinoma with HER2 expression (n = 78)

Clinicopathological parameters	HER2 positive	HER2 negative	P value
<b>Age</b>			
< 50 years (17)	06 (35.29%)	11 (64.71%)	0.57
$\geq$ 50 years (61)	15 (24.59%)	46 (75.41%)	
<b>Sex</b>			
Male (53)	14 (26.42%)	39 (73.58%)	0.89
Female (25)	07 (28.00%)	18 (72.00%)	
<b>Location</b>			
GEJ (06)	02 (33.33%)	04 (66.67%)	0.13
Proximal stomach (15)	07 (46.67%)	08 (53.33%)	
Distal stomach (57)	12 (21.05%)	45 (78.95%)	

**Table 3:** Correlation between Histologic type of adenocarcinoma and HER2 expression

Type	Total number of cases	HER2 negative n %	HER2 positive n %	X <sup>2</sup>	P value
Intestinal	43	26 (60.47)	17 (39.53)	6.38	0.01
Diffuse	35	31 (88.57)	04 (11.43)		

**Table 4:** Correlation between histologic grade of adenocarcinoma and HER2 expression

Grade	Total no. of cases	HER2 negative n %	HER2 positive n %	X <sup>2</sup>	P value
Grade I	26	15 (57.69)	11 (42.31)	6.25 d.f=2	0.04
Grade II	21	15 (71.43)	06 (28.57)		
Grade III	31	27 (87.10)	04 (12.90)		

**Table 5:** Helicobacter pylori positivity in gastric adenocarcinoma

H.pylori	Total number of cases	Percentage (%)
Positive	19	24.36
Negative	59	75.64

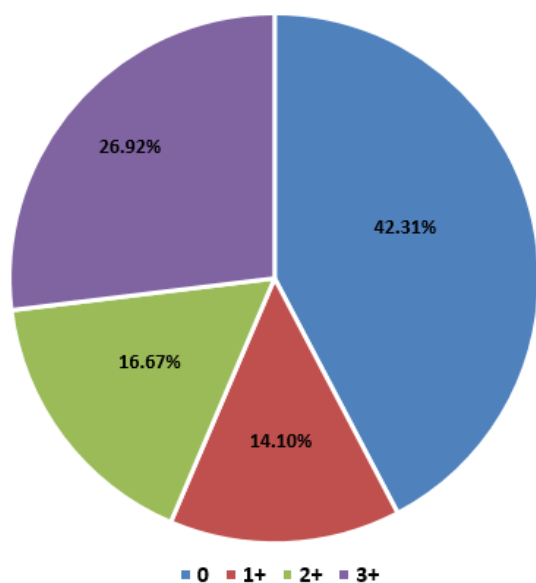
**Table 6:** Correlation between H.pylori positivity in adenocarcinoma and HER2 expression

H.pylori	Total no. of cases	HER2 negative n %	HER2 positive n %	X <sup>2</sup>	P value
Positive	19	13 (68.42)	06 (31.58)	0.05 d.f=1	0.82
Negative	59	44 (74.58)	15 (25.42)		

**Table 7:** Comparison of HER2/neu positivity in present study with other studies

S. N o	Authors	Year	Sample size	HER2 positivity
1	H R Raziee et al <sup>10</sup>	2007	100	26%
2	Laboissire RS et al <sup>11</sup>	2015	124	10.5%
3	Ghosh P et al <sup>12</sup>	2016	54	22.22%
4	AS Nadaf et a <sup>13</sup>	2018	70	23%
5	Abdel Salam et al <sup>14</sup>	2018	76	54%
6	Present study	2019	78	26.92%

HER2 expression in gastric adenocarcinoma (n=78)

**Fig. 1:** HER2/neu expression in gastric adenocarcinoma (n = 78), HER2/neu positive (score 3+ = 26.92%)

with variables like tumor depth (T), lymph node status (N), lymphovascular invasion (LVI) and perineural invasion (PNI). As our sample size was very small, no statistically significant correlation was found between any of these variables.

## 5. Discussion

Gastric carcinoma constitutes a significant health problem worldwide with a high mortality possibly due to late stage presentation and diagnosis. HER2 is among the new markers currently used for early evaluation and treatment of gastric adenocarcinoma. Slamon et al in 1987 proposed that overexpression of HER2 is seen in 20% of breast and in some ovarian and gastric carcinomas. It confers worse biological behavior and clinical aggressiveness in breast cancer.<sup>15</sup>

In our study, the age of patients ranged from 22-87 years with a mean age of  $60.08 \pm 13.55$  years. For statistical purpose, cases were grouped into <50 years and  $\geq 50$  years.

The expression of HER2 was higher in patients of < 50 years of age. Our finding was similar with that of Nadaf et al.<sup>13</sup>

In the present study, percentage of gastric adenocarcinoma was found more in males than in females but HER2 positivity was slightly higher in females (28%) than in males (26.42%). There was no significant correlation of HER2 with age and sex of patients. Nadaf et al found that adenocarcinoma was more common in male but HER2 positivity were more in males than in females, which was non concordant with our study.<sup>13</sup>

In our study, most common location of adenocarcinomas were in distal stomach (73.08%). However, it was found that HER2 positivity was more in tumors located in proximal stomach. Statistical analysis showed no significant correlation between location of tumor and HER2 positivity. Nadaf et al also divided their cases according to location as GEJ, proximal and distal stomach but HER2 positivity was more in tumor located in GEJ.<sup>13</sup>

Lauren's intestinal type was the more common histologic type of adenocarcinomas (55.13%) in the present study. HER2 positivity was also more in intestinal type (39.53%). Considering grades of tumor HER2 positivity was more in grade I (42.31%). There was significant correlation between HER2 and histologic grade (p value= 0.04) and histologic type (p value= 0.01). Raziee et al studied HER2 expression in gastric carcinomas cases and their study was similar to our finding (Table 7).<sup>10</sup>

Ghosh et al in a similar study found that HER2 overexpression was associated with poorly differentiated carcinoma (p=0.0159) and intestinal type of gastric cancer (p= 0.0245). Their study showed concordance with our study with reference to histologic type but not with histologic grade.<sup>12</sup> Abdel Salam et al found HER2 positivity more in intestinal type and in grade I tumor.<sup>14</sup> They got statistical correlation between HER2 expression and Lauren intestinal type which was similar with our study.

Hence the role of HER2 overexpression as good or bad prognostic factor still remains controversial and more studies with a large sample size are required to establish its role in gastric adenocarcinoma.

In present study H.pylori was positive in 24.36% of cases of adenocarcinomas. HER2 positivity was 31.58% in H.pylori positive cases and 25.42% in H.pylori negative cases. There was no significant correlation between HER2 expression and presence of H.pylori (Table 6). Ghosh

et al<sup>12</sup> found that only 2 out of 12 cases of HER2 positivity were positive for H.pylori and there was no significant correlation. Their study was similar with our study. Sreeram et al<sup>16</sup> found that HER2 expression had a negative correlation with density of H.pylori colonization and was statistically significant (p value= 0.020). Our finding was non concordant with their study.

Table 7 shows the comparison of our study with other studies.

In the present study, the percentage of HER2 positivity in gastric adenocarcinoma by immunohistochemistry could have been higher if the score of 2+ (equivocal) could have been confirmed by FISH/CISH. The equivocal staining score of 2+ has been considered as negative in our study.

## 6. Conclusion

HER2 overexpression in the present scenario is mostly utilised in different types of cancer for targeted therapy. It is no more used as a prognostic factor as previously thought. Nowadays trastuzumab therapy is added in gastric carcinoma case with HER2 positivity. In the present study HER2 was found to be associated with Lauren intestinal type and well differentiated gastric adenocarcinoma. H.pylori though present in some of gastric carcinoma cases with HER2 positivity had no statistical significance. Therefore this study of HER2 in gastric carcinoma will be helpful in the treatment of patients than in deciding the prognostic significance.

## 7. Source of Funding

None.

## 8. Conflict of Interest

None.

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