Original Research Article

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

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A B S T R A C T

Background: Gastric cancer is the 5th most common malignancy and 3rd 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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factor receptor family. Accurate evaluation of expression of HER2 protein can be a help to identify eligible candidates for new targeted therapy. 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. 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.

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 accoring 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.

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 ± 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 Laurens’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

| 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 ≥ 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 ≥ 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 ≥ 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 |
|--------------------------------|---------------|---------------|---------|
| Age                            |               |               |         |
| < 50 years (17)                | 06 (35.29%)   | 11 (64.71%)   | 0.57    |
| ≥ 50 years (61)                | 15 (24.59%)   | 46 (75.41%)   |         |
| Sex                            |               |               |         |
| Male (53)                      | 14 (26.42%)   | 39 (73.58%)   | 0.89    |
| Female (25)                    | 07 (28.00%)   | 18 (72.00%)   |         |
| Location                       |               |               |         |
| GEJ (06)                       | 02 (33.33%)   | 04 (66.67%)   |         |
| Proximal stomach (15)          | 07 (46.67%)   | 08 (53.33%)   | 0.13    |
| 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 % | X² | 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 % | X² | 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 % | X² | 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. No | Authors                     | Year | Sample size | HER2 positivity |
|-------|-----------------------------|------|-------------|-----------------|
| 1     | H R Raziee et al           | 2007 | 100         | 26%             |
| 2     | Laboissiere RS et al        | 2015 | 124         | 10.5%           |
| 3     | Ghosh P et al              | 2016 | 54          | 22.22%          |
| 4     | AS Nadaf et al             | 2018 | 70          | 23%             |
| 5     | Abdel Salam et al          | 2018 | 76          | 54%             |
| 6     | Present study              | 2019 | 78          | 26.92%          |

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.15

In our study, the age of patients ranged from 22-87 years with a mean age of 60.08 ± 13.55 years. For statistical purpose, cases were grouped into <50 years and ≥ 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.13

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 males but HER2 positivity were more in males than in females, which was non concordant with our study.13

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 tumors located in GEJ.13

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).10

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.12 Abdel Salam et al found HER2 positivity more in intestinal type and in grade I tumor.14 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. 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. Srerem et al. found that HER2 expression had a negative correlation with the 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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