Expression of Her2-Neu in Primary Gastric and Gastroesophageal Adenocarcinoma: An Experience from a Tertiary Center in South India

Aditi Damle1 Roopa Rachel Paulose1 Divya Saikumar2 Divya Ail1 Niveditha Kartha3 Renjitha Bhaskaran3 Wesley Jose4 Vidhya Jha5

1 Department of Pathology, Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala, India
2 Department of Pathology, Medical School, Amrita School of Medicine and Research Centre, Kochi, Kerala, India
3 Department of Biostatistics, Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala, India
4 Department of Medical Oncology, Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala, India
5 Department of Cytogenetics, Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala, India

Address for correspondence Roopa Rachel Paulose, FRCPath, CCT (UK), Department of Pathology, Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala 682041, India (e-mail: roopa.paulose@gmail.com; roopapaulose@aims.amrita.edu).

South Asian J Cancer

Abstract

Gastric cancer is one of the most commonly occurring cancers worldwide, often presenting at an advanced stage. Combining targeted therapy with chemotherapeutic agents can enhance and extend the survival of these patients. This 4-year retrospective study aims to assess the prognostic role of Her2-Neu expression in gastric and gastroesophageal (GE) cancer. Clinicopathological features, histological type (Lauren classification) of adenocarcinoma, and Her2 immunohistochemical expression were correlated with disease-free and overall survival in 114 patients. A Her2 score of 0 and 1+ indicated negativity, while 3+ marked positivity. For cases with a 2+ score, fluorescent in situ hybridization (FISH) was conducted for definitive categorization. Statistical analysis employed IBM SPSS version 20.0 software. Among 114 patients, 13 displayed strong Her2-Neu immunopositivity (3+), 9 scored 2+, and 92 were negative (0 [89] and 1+ [3]). FISH classified 4 and 5 cases as positive and negative, respectively. Most (64.7%) Her2-Neu-positive tumors occurred in the proximal stomach/GE junction (GEJ) and exhibited intestinal morphology (94.1%) with moderate differentiation (p-value < 0.05). Notably, 76.5% of Her2-Neu-positive patients exhibited advanced-stage disease with nodal/distant metastasis. The average disease-free survival was 15.4 months (standard error: 3.55) for positive Her2-Neu expression and 22.07 months (standard error: 1.364) for negative expression. The mean overall survival was 21.14 months (standard error: 3.702) for positive expression and 23.91 months (standard error: 1.474) for negative expression. Her2-Neu expression in gastric/GEJ adenocarcinomas correlates with reduced survival. Evaluating HER2-NEU in proximal gastric/GEJ cancers displaying low-grade intestinal morphology serves as both a predictive and prognostic indicator.

Keywords
- gastric cancer
- gastroesophageal adenocarcinoma
- Her2-Neu
- targeted therapy
- trastuzumab


How to cite this article: Damle A, Paulose RR, Saikumar D, et al. Expression of Her2-Neu in Primary Gastric and Gastroesophageal Adenocarcinoma: An Experience from a Tertiary Center in South India. South Asian J Cancer 2023;00(00):00–00

© 2023. MedIntel Services Pvt Ltd. All rights reserved.
This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (https://creativecommons.org/licenses/by-nc-nd/4.0/)

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India
**Introduction**

Gastric/gastroesophageal (GE) carcinoma is one of the common causes for cancer-related mortality in India and worldwide. The Global Burden of Disease Study 1990–2016 observed that gastric cancer is one of the top 10 common cancers in India and accounts for 9% of the total cancers disability-adjusted life years.\(^1\)–\(^4\) The incidence of gastric cancer is higher in South India\(^5\) and often presents at advanced stages. The mainstay of treatment is surgery with perioperative chemotherapy.

The role of various cell surface antigens/transmembrane proteins/growth factor receptors like CDH1, MCT1, and MCT4\(^6\)–\(^7\) have been studied in gastric cancer. Her2-Neu oncogene is a member of the erbB-like oncogene family which are physiologically involved in signal transmission and are constituted by family of four cell surface receptors—HER1, HER2, HER3, and HER4, which exist as monomers and heterodimerize with her2 on ligand binding, in turn generating strong intracellular signals controlling normal cell proliferation.\(^3\)–\(^9\) Hence, Her2 overexpression in neoplastic cells causes uncontrolled cancer cell proliferation.\(^5\)

The role of expression of Her2-Neu in gastric/GE junction (GEJ) cancers have gained interest after witnessing improved outcome on targeted therapy with trastuzumab (recombinant human anti-her2 monoclonal antibody).\(^3\)–\(^11\)

Despite being a poor prognosticator, it has been proved promising in early gastric cancers when used in combination with other chemotherapeutic agents.\(^10\)–\(^12\)–\(^16\)

The immunohistochemical expression of Her2-Neu in gastric/GE cancers displays heterogeneity and also varies geographically.\(^11\)–\(^18\)

As there is paucity of Indian data on this, we conducted this study to evaluate the role of expression of Her2-Neu in gastric/GE cancer as a prognostic marker.

**Materials and Methods**

This is a 4-year (January 2011–December 2014) retrospective study followed by 7 years of follow-up (i.e., 2015–2021) of patients who underwent surgical resection for primary gastric or GEJ adenocarcinoma, at a tertiary center in South India. Institutional ethics committee approval was obtained for the study.

Details regarding patient demographics, tumor site and stage, and treatment including neoadjuvant/adjuvant therapy were obtained from the hospital information system. All cases were histologically categorized as per Lauren’s classification into intestinal or diffuse and staged as per TNM 8th Edition. Immunohistochemistry (IHC) with Her2-Neu monoclonal primary antibody (polyclonal rabbit anti-human c-erbB-2 oncoprotein [DAKO] with a dilution of 1:600) was performed on 3-μm thick deparaffinized sections of tumor tissue using the two-step Super Sensitive Polymer HRP IHC detection kit including positive and negative controls in each run. Her2-Neu expression in the neoplastic cells was evaluated using previously validated scoring system adapted from Hofmann et al\(^19\) and Rüschoff et al.\(^14\) Score of 0 and 1+ was considered as negative, while 3+ was considered as positive. Score 2+ was considered as equivocal/borderline and further assessed by fluorescent in situ hybridization (FISH) for definitive categorization.

**Follow-up period:** The median follow-up period was 2 years (range 1–7 years). Information regarding disease progression/overall survival was obtained from hospital case notes and/or telephonie contact/cancer registry data.

Statistical analysis was performed using IBM SPSS version 20.0 software program (SPSS Inc., Chicago, Illinois, United States). Pearson’s chi-square test was used to analyze the association of Her2-Neu expression with clinical details, type, and differentiation of adenocarcinoma and pathological stage of the tumor. To find the probability of overall survival and disease-free survival, Kaplan–Meier analysis was done and followed by log rank test for the comparison. A p-value of <0.05 was considered to be statistically significant.

**Results**

A total 114 patients of histologically proven gastric/GE adenocarcinoma was considered in the study with a mean age of 61.75 ± 12.07. There were 90 males (78.95%) and 24 females (21.05%); male-to-female ratio was 3.75:1. The median age of the patients was 64.5 years (range 22–80 years). Eighty-two percent of gastric cancer patients were older than 50 years of age. The most common location of the tumor was the gastric cardia and GEJ (42.9%). The predominant histological type of adenocarcinoma was intestinal subtype (83.3%). Fifty-two percent of the tumor was of adenocarcinoma intestinal subtype (83.3%). Fifty-two percent of the tumor was of

1. 1. Tumors with Her2-Neu positivity (n = 17)

The tumors with positive Her2-Neu expression were more frequently located in the proximal stomach (cardia) and GEJ (64.7%), with a predominant intestinal morphological pattern (94.1%) and well/moderate differentiation (grade 1 and 2; 82.35%).

The comparison of histological differentiation with Her2-Neu expression was found to be statistically significant (p-value 0.005) (\(\text{Fig. 2}\)). Diffuse morphology was seen in only one patient. Seventy-five percent of patients had advanced stage of disease with metastases (\(\text{Table 3}\)).

2. 2. Tumors showing Her2-Neu negative (n = 97) had no specific site location, histology, grade, or stage bias. In univariate analysis there were found to be no statistically significant association for tumor attributes with Her2-Neu expression status (\(\text{Table 3}\)).

**Follow-Up**

The mean and median follow-up period was 2.6 and 3 years, respectively. Eight patients with Her2-Neu positive (2) and Her2-Neu negative (6) were lost to follow-up. Fifty-six
patients among 106 (52.8%) in our study population had disease recurrence and 51 (51%) died of the disease. Eleven Her2-Neu positive patients (73.3%) had disease recurrence and 9 (69.2%) died of the disease. In comparison, with negative expression for Her2-Neu protein, 45 (49.5%) had disease recurrence and 42 (48.28%) died of the disease (Fig. 3). The comparison of mortality and recurrence status was not found to be statistically significant (p-value 0.086 and 0.159, respectively).

The overall mean disease-free survival for our study population was 21.15 months with standard error of 1.498 (95% confidence interval [CI]: 18.216–24.087). The overall mean disease-free survival for the patients showing positive
Her2-Neu expression was 15.4 months with standard error of 3.55 and for patients with negative Her2-Neu expression was 22.07 months with standard error of 1.364 (p-value 0.08) (Fig. 4).

The mean overall survival for our study population was 23.51 months with standard error of 1.364 (95% CI: 20.841–26.189). The mean overall survival for the patients showing positive Her2-Neu expression was 21.14 months with standard error of 3.702 and for patients with negative Her2-Neu expression was 23.91 months with standard error of 1.474 (p-value 0.245) (Fig. 5). As the number of patients with Her2 positive disease were low, multivariate analysis was not feasible.

### Table 3

Multivariate table showing comparison of tumor attributes/characteristics with Her2-Neu expression status

<table>
<thead>
<tr>
<th>Variable</th>
<th>HER2-NEU positive (total 17)</th>
<th>HER2-NEU negative (total 97)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Age at diagnosis years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 50 years</td>
<td>15</td>
<td>88.24</td>
<td>79</td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>2</td>
<td>11.76</td>
<td>18</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>82.35</td>
<td>76</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>17.65</td>
<td>21</td>
</tr>
<tr>
<td>Site of tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEJ</td>
<td>11</td>
<td>64.71</td>
<td>38</td>
</tr>
<tr>
<td>Gastric body/fundus</td>
<td>2</td>
<td>11.76</td>
<td>35</td>
</tr>
<tr>
<td>Distal stomach</td>
<td>4</td>
<td>23.53</td>
<td>24</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal type</td>
<td>16</td>
<td>94.12</td>
<td>79</td>
</tr>
<tr>
<td>Diffuse type</td>
<td>1</td>
<td>5.88</td>
<td>18</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well/moderate</td>
<td>14</td>
<td>82.35</td>
<td>41</td>
</tr>
<tr>
<td>Poor</td>
<td>3</td>
<td>17.65</td>
<td>56</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/T2</td>
<td>4</td>
<td>23.53</td>
<td>20</td>
</tr>
<tr>
<td>T3/T4 (advanced stage)</td>
<td>13</td>
<td>76.47</td>
<td>77</td>
</tr>
<tr>
<td>Node/distant metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>13</td>
<td>76.47</td>
<td>69</td>
</tr>
<tr>
<td>Absent</td>
<td>4</td>
<td>23.53</td>
<td>28</td>
</tr>
</tbody>
</table>

Abbreviation: GEJ, gastroesophageal junction.

### Fig. 3

Frequency of disease recurrence in relation to Her2-Neu expression.

### Discussion

The incidence of gastric cancer in India is relatively low compared with other countries. However, a regional variation has been observed in India with the highest incidence recorded from Southern and Eastern India.20 There is paucity of data from these regions and hence this study was undertaken to understand the clinicopathological characteristics and survival outcome of patients with gastric and GEJ adenocarcinoma in relation to the expression of Her2-Neu, a potential oncological target. Concordant with other Indian studies,21,22 this study found a male gender preponderance...
Expression of Her2-Neu in Primary Gastric and Gastroesophageal Adenocarcinoma  Damle et al.

Asian countries like Japan and Korea.

Frequency of positivity for Her2-Neu in gastric/GEJ cancers has been found variable across continents. Lei et al\(^\text{15}\) in their meta-analysis have stated a slightly higher rate of positivity in Asian countries, that is, 19.52% in comparison to the European countries which is 16.91%. This heterogeneous expression has been observed between Asian countries and even within the Indian subcontinent. Shan et al\(^\text{29}\) in a Chinese study demonstrated a frequency of 9.8%, while Matsusaka et al\(^\text{10}\) in a Japanese multicenter observational study demonstrated 15.6% frequency of high Her2-Neu expression. Among the Indian studies, a geographic variation has been observed with Sukanya et al\(^\text{22}\) from Tamil Nadu reporting a 12% frequency, Patil et al\(^\text{31}\) in their multicentric clinical trial showed 7% frequency and a systematic review of 41 studies reported a median of 15%\(^,\text{32}\) Gupta et al\(^\text{21}\) from Delhi showed 24.5% frequency, Panda et al\(^\text{33}\) from the state of Orissa showed 18.7% frequency, and the frequency in the present study done in a tertiary care institute of Kerala was 15%.

Significant association between Her2-Neu expression and proximal gastric/GEJ tumor site has been observed in studies done by Roy et al\(^\text{23}\) Shan et al\(^\text{29}\) and Lei et al\(^\text{15}\) and in the present study. Although Sukanya et al\(^\text{22}\) demonstrated higher expression of Her2-Neu in distal gastric cancers, the number of GEJ tumors was low in their comparatively smaller study population (4 out of total 70). A significant association of Her2-Neu expression with well/moderately differentiated (grade 1 and 2) intestinal type adenocarcinoma is there in the present study and in those conducted by Gupta et al\(^\text{21}\) Panda et al\(^\text{33}\) Lei et al\(^\text{15}\) and Shan et al\(^\text{29}\).

GEJ tumors are more aggressive and with advanced stage of disease\(^,\text{24}\). In the present study, both Her2-Neu positive and Her2-Neu negative groups showed distant/nodal metastasis with only a borderline difference between them, not statistically significant. Lei et al\(^\text{15}\) and Panda et al\(^\text{33}\) in their meta-analysis and study, respectively, have demonstrated a statistical correlation between Her2-Neu positive tumor status and advanced tumor staging.

There is limited Indian data on survival/outcome of patients with Her2-Neu expression on gastric cancer; hence, this index study seems to be one of its kind. The mean overall disease-free survival and overall survival for the patients showing positive Her2-Neu expression in tumor was lower in comparison to those with negative Her2-Neu tumor expression in the present study; despite administration of neoadjuvant therapy (to 35 and 42% patients with Her2-Neu positive and negative expression in tumor, respectively) and adjuvant chemotherapy (to 70% patients from each group). Trastuzumab was not given to any of the patients in the present study done at our center, due to the high cost of the drug in the given period of study. Chua and Merrett\(^\text{28}\) in their review of 49 studies comprising 11,337 patients found that in patients with positive Her2-Neu expression the median 3-year disease-free survival was 58% (range: 50–88%) and in those with negative Her2-Neu expression the median 3-year disease-free survival was 86% (range: 62–97%); the median overall survival and 5-year survival rate was 21 (42%) and 33 months (52%) in Her2-Neu positive and Her2-Neu negative groups, respectively.

The fact that Her2-Neu positive tumors demonstrate advanced disease with lower survival rates despite giving neoadjuvant and adjuvant chemotherapy, targeted interventions are a ray of hope.

Fig. 4 Kaplan–Meyer curve depicting comparison of overall disease-free survival between Her2-Neu positive and negative groups.

Fig. 5 Kaplan–Meyer curve depicting comparison of overall survival between Her2-Neu positive and negative groups.
Consideration of conversion surgery in primary metastatic gastric cancer. The outcomes were compared to 47 Her2-Neu positive patients with recurrent or primary metastatic gastric cancer. The outcomes were comparable to that of the ToGA trial, and also concluded that the consideration of conversion surgery in fit patients can be done with R0 resection. After observing significantly improved survival on the use of dual-targeted anti-Her2-Neu therapy in breast cancer, Tabernero et al had designed a study—JACOB—to assess the efficacy and safety of pertuzumab plus trastuzumab and chemotherapy in patients with previously untreated Her2-positive metastatic gastric/GEJ cancers. The primary results showed that the addition of pertuzumab did not significantly improve overall survival at ≥24.4 months’ median follow-up, possibly due to increased complexity of gastric cancer and multifactorial disease progression. However, descriptive end-of-study results showed some but limited evidence of treatment activity and acceptable toxicity profile for the use of above combination.

Conclusion
Expression of Her2-Neu in gastric/GEJ adenocarcinomas though low in our population, was associated with lower survival and found to be a poor prognosticator.

Note

Availability of Data and Material
The authors confirm that the data supporting the findings of this study are available within the article in form of tables.

Ethics Approval
Obtained.

Authors’ Contribution
The study’s conception and design saw significant input from R.R.P., A.D., and V.J. A.D., D.A., and D.S. were responsible for data acquisition. Data analysis and interpretation were carried out by A.D., R.R.P., N.K., and R.B. A.D., R.R.P., and W.J. were involved in drafting and revising the article for substantial intellectual content. The final version approval for publication rests with R.R.P.

Funding
Funding was provided by the Amrita Institute of Medical Sciences, Kochi for performing Immunohistochemistry and FISH on the cases.

Conflict of Interest
None declared.

Acknowledgment
The authors thank the technical staff – Pathology Department, Amrita Institute of Medical Sciences, Kochi.

References
16 Bang YJ, Van Cutsem E, Feyereislova A, et al; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376
Expression of Her2-Neu in Primary Gastric and Gastroesophageal Adenocarcinoma


Shan L, Ying J, Lu N. HER2 expression and relevant clinicopathological features in gastric and gastroesophageal junction adenocarcinoma in a Chinese population. Diagn Pathol 2013;8:76


Patil PS, Mehta SA, Mohandas KM. Over-expression of HER2 in Indian patients with gastric cancer. Indian J Gastroenterol 2013;32(05):350

Chua TC, Merrett ND. Clinicopathologic factors associated with HER2-positive gastric cancer and its impact on survival outcomes—a systematic review. Int J Cancer 2012;130(12):2845–2856


