HER 2 Expression in Gastric and Gastro-esophageal Junction (GEJ) Adenocarcinomas

ABSTRACT

Introduction: Gastric cancer is one of the leading causes of cancer mortality in the world/India with majority being diagnosed at an advanced stage. Various chemotherapeutic regimens have modestly improved overall survival leading to quest for novel therapeutic agents. Overexpression of HER2 in many gastric cancers has lead to the advent of targeted therapy with anti HER2 antibody like Trastusumab which has improved the overall survival.

Materials and Methods: Sixty cases of gastric adenocarcinomas (44 biopsies and 16 gastrectomies) over the past five years (June 2009 to June 2014), were included in the study. Diagnosis was confirmed by review of slides and IHC with anti HER2 antibodies was performed using Dako Real Envision Detection system and scoring was done by Hoffmann et al., scoring system.

Results: Of the 60 cases, majority were males (60%), with a mean age of 65.65 yrs. Tumours in antrum (76.7%) formed the major bulk. HER2 expression was observed in 26.7% of Tumours, predominantly in males (p=0.006) and intestinal type (p= 0.054). HER2 expression correlated with Tumour grade (moderately differentiated and well differentiated, p= 0.042). Tumours of gastro-esophageal junction (GEJ) showed HER2 expression in 45.5% as opposed to 22.4% in gastric location. Poorly differentiated and diffus type of adenocarcinomas did not express HER2. Two of three Tumours from patients in the age group 31-40 y expressed HER2.

Conclusion: Male gender, intestinal-type and moderately differentiated gastric cancers may be the ones that can be targeted for therapy using Herceptin. Though trastusumab is approved for advanced gastric and GEJ cancers, its role in adjuvant / neo-adjuvant setting in early stages needs to be evaluated with newer agents like Pertuzumab, Bevacizumab, especially in young patients.

INTRODUCTION

Gastric cancer is the 5th most commonly diagnosed cancer and the 3rd most common cause of cancer related death globally [1]. Barrett’s esophagus and dysplasia are associated with the development of esophageal adenocarcinoma [2] while *Helicobacter pylori* infection, atrophic gastritis, intestinal metaplasia, and dysplasia are related with gastric adenocarcinoma [3].

Surgical resection is the mainstay of treatment and can cure patients with early-stage cancer. The survival rate of patients with advanced resectable gastric or gastroesophageal junction (GEJ) cancers, however, remains poor despite new treatment strategies, such as perioperative chemotherapy or adjuvant chemoradiation [4]. Objective response rates range from 10% to 30% for single-agent therapy and 30% to 60% for combination regimen [5] along with uncertainty regarding the choice of the chemotherapy regimen [6]. New rationally designed molecular targeted therapies are urgently needed which interfere with the signalling cascades involved in cell differentiation, proliferation and survival.

The HER2 protein (p185, HER2/neu, ErbB-2) is a 185-kDa transmembrane tyrosine kinase (TK) receptor and a member of the epidermal growth factor receptor (EGFR) family. HER2 is encoded by a gene located on chromosome 17q21. In carcinomas, HER2 acts as an oncogene, mainly because high-level amplification of the gene induces protein overexpression in the cellular membrane and subsequent acquisition of advantageous properties for a malignant cell [7]. Evaluated extensively in breast cancers, the role of HER 2 over-expression in gastric adenocarcinomas is being studied. With few reports from India [8-10] and none from southern part of India, we attempted to study the same at a medical college hospital based in Bengaluru.

KEYWORDS: Gastric / GEJ adenocarcinoma, HER2/neu, Immunohistochemistry (IHC)

AIMS AND OBJECTIVES

- To examine the frequency of expression of HER2 in primary gastric and gastro-esophageal junction(GEJ) adenocarcinoma.
- To find its correlation with clinic-pathological parameters like age, sex, location of Tumour, histological grade, Lauren classification, p TNM staging and *H. pylori* status.

MATERIALS AND METHODS

This was an observational study including all histopathologically diagnosed adenocarcinomas of stomach and gastro esophageal junction (GEJ) which were diagnosed in the Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru over a period of five years (from June 2009 to June 2014).

Relevant clinical details were collected from the patients’ and case files in prospective cases (29 cases), while available details were collected from the medical records in retrospective cases (31 cases). Radiological and endoscopy details were retrieved wherever possible. All gastrectomy/gastric biopsy specimens were fixed in 10% neutral buffered formalin. Tissues were processed, embedded in paraffin, sections were taken and stained with routine H&E. Histopathological diagnosis was made and adenocarcinomas were classified as intestinal / diffuse according to Lauren classification. Giemsa staining was performed for *H. pylori* status. Specimens which were tiny, not fixed were excluded from the study.

In retrospective cases (31 cases), slides were reviewed to confirm diagnosis. Respective paraffin blocks ( both from prospective and retrospective cases) were retrieved, 4 µ sections were taken and mounted on poly L lysine coated slides taking care to mount the tissue sections flat and wrinkle free as possible. The slides were placed in the oven at 60°C for 30 min. Antigen retrieval was done
using Citrate buffer at pH 5.5 to 6.0 in a domestic microwave at high power for 5 min. Endogenous peroxidase blocking was done with 3% hydrogen peroxide. Immunohistochemistry was done using Rabbit polyclonal HER 2 antibody in the dilution of 1:600 using Dako REALTM En Vision DetectionTM system, (Peroxidase /DAB+, K5007).

The Immunohistochemistry slides were scored by two pathologists individually according to scoring system by Hoffman et al., [Table/Fig-1]. All cases with score 3+ were considered positive.

<table>
<thead>
<tr>
<th>Score</th>
<th>Surgical specimen-staining pattern</th>
<th>Biopsy specimen-staining pattern</th>
<th>HER2 over expression assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No reactivity or membranous reactivity in &lt;10% of Tumour cells</td>
<td>No reactivity or no membranous reactivity in any Tumour cell</td>
<td>Negative</td>
</tr>
<tr>
<td>1+</td>
<td>Faint/barely perceptible membranous reactivity in ≥10% of Tumour cells; cells are reactive only in part of their membrane</td>
<td>Tumour cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of Tumour cells stained</td>
<td>Negative</td>
</tr>
<tr>
<td>2+</td>
<td>Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% of Tumour cells</td>
<td>Tumour cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of Tumour cells stained</td>
<td>Equivocal</td>
</tr>
<tr>
<td>3+</td>
<td>Strong complete, basolateral, or lateral membranous reactivity in ≥10% of Tumour cells</td>
<td>Tumour cell cluster with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of Tumour cells stained</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**STATISTICAL ANALYSIS**

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data were made,

**Assumptions**

1. Dependent variables should be normally distributed,
2. Samples drawn from the population should be random, Cases of the samples should be independent.

Student t-test was used to find the significance of study parameters on continuous scale between two groups on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

**Statistics:**  
* Moderately significant (p-value: 0.01<p<0.05)  
** Strongly significant (p-value: p<0.01)

The Statistical software, SAS 9.2, SPSS 15.0, Stata 10.1, Med Calc 9.0.1,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

**RESULTS AND OBSERVATION**

The study included 60 proven cases of adenocarcinomas of which, 44 were biopsies and 16 were gastrectomy specimens. Age of patients ranged from 31-85 y. Majority of patients belonged to the age group of 61-70 y. Mean age of the patients was 65.65 y with males accounting for 60% [Table/Fig-2]. Most of the tumours were located in antrum (76.7%) followed by GEJ. According to Lauren classification, 81.7 % of Tumours were of intestinal type and the rest were of diffuse type (18.3%). Moderately differentiated Tumours were the predominant (66.7%) type followed by poorly differentiated (18.3%) and well differentiated type (15%). (p=0.013* Significant, Chi-Square test).

**HER2 scores of patients studied:** Majority of patients was of score 0 (61.7%) while 26.7% of patients showed a score of 3+ suggesting HER 2 positivity [Table/Fig-3-5]. HER2 positivity was more in gastric biopsies (31.8%) as opposed to 12.5% in gastrectomies [Table/Fig-6]. A score of 2+ i.e., equivocal was seen in 5% of cases and 6.7% cases were of score 1+ [Table/Fig-7]. HER2 positivity was found more in the age group of 31-40 y (2 out of 3 cases studied were of score 3+ and one case was equivocal), (p=0.253, NS, Fisher Exact test). HER2 positivity was found more in males (30.6%). Intestinal type appeared to express HER2 in relatively lower percentage of Tumours (32.7 %). Adenocarcinomas of gastric origin expressed HER 2 in 22.4% when compared to the GEJ Tumours (45.5%). None of the diffuse type showed HER2 positivity [Table/Fig-8]. HER2 positivity was found more frequently in moderately differentiated Tumours (37.5%), followed by 11.1% in well-differentiated Tumours

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total number of patients</th>
<th>HER2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>12(50%)</td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>25(69.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>37(61.7%)</td>
</tr>
</tbody>
</table>

**Table/Fig-2:** Gender distribution in the study group  
P=0.006**, significant, Fisher Exact test

**Table/Fig-3:** Moderately differentiated adenocarcinoma showing strong membranous HER2 positivity (score 3+). Case no : 1 (400x, IHC)

**Table/Fig-4:** Well-differentiated adenocarcinoma (gastrectomy) showing strong lateral wall staining on HER2 (score 3+). Case no : 55 (400x, IHC)
HER2 positivity [Table/Fig-10]. We observed in a minority of cases
[Table/Fig-9]. None of the poorly differentiated Tumours expressed
HER2 positivity [Table/Fig-10]. We observed in a minority of cases
intense cytoplasmic positivity in the Tumour cells (6/60 cases). No
correlation was found between HER2 positivity and pathological
stage (pTNM). In our study most patients were of pathological stage
T2 (87.5%) while 1 case each (6.25% each) was of stage T3 and T4.
Regarding the pathologic N stage, only 3 cases (18.75%) were of
N 0 stage, rest were of N 1 stage (81.25%). Distant metastasis was
not documented in any of the cases.

DISCUSSION

The first cases of possible gastric cancer were reported in the Ebers
Papyrus, written in 1600 BC. Benign and malignant gastric ulcers
were only described later by J. Cruveilhier in 1835 demystifying
the death of Napoleon Bonaparte in 1821 who probably had an
extensive scirrhous carcinoma of the stomach, complicated by

The incidence of gastric cancer varies in different parts of the world
with highest incidence rates documented in Eastern Asia, South
America, Eastern Europe [12,13] (fifth most common cancer in
Europe with 159 900 new cases and 118 200 deaths in 2006)
[14]. Linxian, China is known to have one of the highest rates of
oesophageal/gastric cardia cancer in the world [15]. North America
and Africa show the lowest recorded rates [12,13]. In India gastric
cancer is the fifth most common cancer among males [16] (7th in
Karnataka) [17] and seventh most common cancer among females
[16] (8th in Karnataka [17]). According to the population based
cancer registry at Kidwai Memorial Institute of oncology, under
the national cancer registry program of ICMR, which covers the
resident population of Bangalore urban Agglomeration, the most
predominant site of cancer constituting 9% of total cancers among
males is stomach cancer [17]. Improved food hygiene, sanitation,
and food preservation techniques have lead to a decline in the
incidence of gastric cancer all over the world. But in certain parts
of India, gastric cancer in South Indian males has been reported
to be more common and occurring a decade before their North
Indian counterparts. Differences in some dietary pattern and use
of tobacco and alcohol have been considered as potential risk factors
[18].

Many gastric cancer patients present with advanced stage disease,
and the prognosis remains poor. Improvements in the treatment of
gastric cancer, including combination chemotherapy, have resulted
in improved overall survival versus single-agent chemotherapy alone.
Additional therapy aimed at specific targets in cancer has shown a
survival benefit in certain Tumours. One of these cellular targets,
human epidermal growth factor receptor 2 (HER2/neu) protein, a
185-kDa transmembrane tyrosine kinase receptor, is associated
with Tumour proliferation, migration, and differentiation.

The overexpression of HER2/neu on Tumour cells versus normal
cells allows selective targeting of malignant cells with anti-HER2/neu
therapy. HER2/neu overexpression has been identified in a variety
of neoplasms but has been most widely studied in breast cancer.
Approximately 25% to 30% of breast cancers overexpress HER2/
neu. HER2/neu-positive status is associated with more aggressive
disease and is an important predictive factor of response to therapy
with trastuzumab [19].

Recently published data, from the randomized, prospective phase III
clinical trial ToGA provided first documentation of the clinical benefit
of trastuzumab when used in combination with chemotherapy in
the setting of advanced gastric and gastroesophageal junction (GEJ)
cancer. Median overall survival was 13.8 months in trastuzumab
arm compared to 11.1 months in the chemotherapy only arm. As
a result of the survival benefit, laboratories are facing increasing
demand for HER2 tests to determine patient eligibility for targeted
therapy [20]. In addition to trastuzumab several ongoing trials are
testing the efficacy of other targeted agents in gastric cancer.
Lapatinib is a TK inhibitor that targets both HER2 and EGFR and

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total number of patients</th>
<th>HER2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly</td>
<td>11</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Moderately</td>
<td>40</td>
<td>1+ (47.5%)</td>
</tr>
<tr>
<td>Well</td>
<td>9</td>
<td>2+ (88.9%)</td>
</tr>
<tr>
<td>Differentiated</td>
<td>60</td>
<td>3+ (67.1%)</td>
</tr>
</tbody>
</table>

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<th>Grade</th>
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<td>Differentiated</td>
<td>60</td>
<td>3+ (67.1%)</td>
</tr>
</tbody>
</table>
H. pylori associated gastric cancers begins with H. pylori causing intestinal metaplasia moving on to dysplasia and last to malignant transformation [32]. During sampling for diagnosis of malignancy, we always sample the Tumour part, which no longer hosts the H. pylori, contributing to the negative H. pylori status. More so in the case of biopsies, we are studying only the Tumourous part and the possibility of detecting H. pylori is remote. Probably if we had studied an area away from Tumour in case of gastrectomies, we would have been able to detect the presence or absence of H. pylori association. As majority of gastrectomies were retrospective, we were unable to study the mucosa away from Tumourous area.

One of the drawbacks of our study is that we were not able to confirm IHC 1+ and 2+ cases by FISH. Studies have shown excellent IHC-FISH concordance (>95%) in IHC 0 and IHC 3+ cases, suggesting that these IHC scores may not require routine FISH confirmation. Reliable separation of IHC 1+ and IHC 2+ patterns is challenging in small biopsy specimens, due to crush and edge artifacts [30]. Reliable separation of IHC 1+ and IHC 2+ patterns is challenging in small biopsy specimens, due to crush and edge artifacts [30].

Preliminary data from TOGA trial showed that patients with ampliﬁed Tumours without overexpression (IHC 0 and 1+ cases) did not show substantial overall survival beneﬁt from trastuzumab. Still we may have missed some of the IHC 2+ cases which would have shown HER2 ampliﬁcation by FISH [21]. Another pitfall of IHC that we noticed was that normal foveolar epithelium showed non speciﬁc cytoplasmic staining. Though there was clear distinction between

### Table/Fig-10: Comparison of Her 2 expression in gastric cancers reported by various authors with our study [7-10,25]

<table>
<thead>
<tr>
<th>Author</th>
<th>No of cases studied</th>
<th>Geographic zone</th>
<th>HER2 expression by IHC in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yano et al., [7]</td>
<td>n= 200</td>
<td>Japan</td>
<td>23</td>
</tr>
<tr>
<td>Gravalos et al., [7]</td>
<td>n= 166</td>
<td>Europe</td>
<td>13</td>
</tr>
<tr>
<td>Park et al., [25]</td>
<td>n= 182</td>
<td>Korea</td>
<td>16</td>
</tr>
<tr>
<td>Lordick et al., [7]</td>
<td>n= 1527</td>
<td>Europe, Asia, Latin America</td>
<td>22</td>
</tr>
<tr>
<td>Shekharan A et al., [8]</td>
<td>n= 52</td>
<td>India</td>
<td>44.2</td>
</tr>
<tr>
<td>Prachi S Patil et al., [9]</td>
<td>n= 43</td>
<td>India</td>
<td>7</td>
</tr>
<tr>
<td>Mallika Tiwari et al., [10]</td>
<td>n= 70</td>
<td>North India (Varanasi)</td>
<td>21.4</td>
</tr>
<tr>
<td>Our Study (2014)</td>
<td>n= 60</td>
<td>India</td>
<td>26.7</td>
</tr>
</tbody>
</table>

### Table/Fig-11: Comparison of HER2 expression in gastric cancers reported by various authors based on tumor location and histological type with our study [7,10,23]

<table>
<thead>
<tr>
<th>Author</th>
<th>Histologic type</th>
<th>Localisation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intestinal</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Tanner et al., [7]</td>
<td>21.5 (n=65)</td>
<td>2 (n=46)</td>
</tr>
<tr>
<td>Gravalos et al., [7]</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Lordick et al., [7]</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>Shank et al., [23]</td>
<td>16.8 (n=650)</td>
<td>2.3 (n=564)</td>
</tr>
<tr>
<td>Mallika Tiwari et al., [10]</td>
<td>45 (n=11)</td>
<td>12 (n=44)</td>
</tr>
<tr>
<td>Our Study</td>
<td>32.7 (n=49)</td>
<td>0 (n=11)</td>
</tr>
</tbody>
</table>
normal epithelium and Tumour cells and this did not interfere with our interpretation, one need to be careful during interpretation of HER2 staining. Intense cytoplasmic positivity (probably related to cytoplasmic mucin) in the Tumour cells though considered non-specific in the study, needed FISH confirmation because strong cytoplasmic staining can mask membranous positivity [30].

CONCLUSION
An accurate assessment of HER2 expression in gastric cancer patients is of importance and utility in the optimal selection of patients for Trastuzumab (Herceptin) therapy. Our study found an HER2 overexpression of 22.4% in gastric cancers similar to most studies in India and rest of the world. We found a statistically significant correlation of HER2 overexpression with male gender, intestinal-type and moderately differentiated gastric cancers suggesting that these may be the candidates for targeted therapy using Herceptin. Additional studies are needed to explore the role of HER2 as an independent prognostic factor. Also diffuse type of gastric cancers not expressing HER2 needs to be studied, to confirm any existing geographic variation. Though Herceptin is approved for advanced gastric and GEJ cancers, role of herceptin in adjuvant / neo-adjuvant setting in early stages needs to be evaluated with newer agents like Pertuzumab, Bevacizumab, especially in young patients.

REFERENCES


