Study of Human Epidermal Growth Factor Receptor 2 (HER2) Expression in Gastric Carcinoma

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ABSTRACT

BACKGROUND

Gastric carcinoma is an important cause of cancer related mortality worldwide. Majority of the patients are diagnosed in the advanced stage of the disease. The main treatment modalities are surgery and chemotherapy, but the survival rate of patients with advanced resectable gastric cancer remains poor. For patients with unresectable gastric cancer, chemotherapy remains the treatment of choice. Into this scenario comes the importance of newer targeted therapeutic agents which improve survival rates with acceptable toxicity effects. HER2 is a growth factor implicated in disease initiation and progression, and its expression is associated with a poor prognosis. The aim of this study is detection of HER2 expression in gastric carcinoma and evaluate its relationship with the histopathological characteristics. This would be the stepping stone for patients with tumours that are HER2 positive who could benefit from targeted therapeutical agents like Trastuzumab.

METHODS

Gastrectomy specimens which were diagnosed as Gastric Carcinoma in the Department of Pathology, Government Medical College, Trivandrum, during a period of two years were included in this study. Routine Haematoxylin and Eosin staining and immunohistochemistry for HER2 were done.

RESULTS

Thirty eight cases of gastric carcinoma were received during the study period. Intestinal type adenocarcinoma formed the bulk of the tumours (68.42 %), followed by the diffuse type adenocarcinoma (18.42 %). Of the 38 cases, 10 cases showed HER2 positivity. All the positive cases were intestinal type of adenocarcinomas.

CONCLUSIONS

Our study concluded that 26 % of gastric carcinomas showed positive immunoreaction for HER2 and HER2 overexpression was more in intestinal type adenocarcinomas. HER2 overexpression was also associated with higher stage tumours. There was no association with the patient's age, gender, location of tumour and tumour differentiation.

KEYWORDS

Gastric Carcinoma, HER2 expression, Immunohistochemistry, Lauren Classification

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DOI: 10.18410/jebmh/2020/564

How to Cite This Article:
Lilly LVN, Sukumaran G. Study of human epidermal growth factor receptor 2 (HER2) expression in gastric carcinoma. J Evid Based Med Healthc 2020; 7(47), 2747-2751. DOI: 10.18410/jebmh/2020/564

Submission 23-08-2020,
Peer Review 02-09-2020,
Acceptance 22-07-2020,
Published 23-11-2020.

BACKGROUND

Gastric carcinoma is the fourth most common cancer in the world, accounting for 7.8 % of all cancers worldwide. It remains the second leading cause of cancer death. The highest incidence of this deathly tumour is reported in East Asia, Eastern Europe, Central and Latin America.[1] The initial classification of gastric carcinoma into intestinal and diffuse type adenocarcinoma was made as early as 1965.[2] The intestinal type adenocarcinoma was the predominant tumour type among patients over 50 years of age and the diffuse type was more prevalent among patients below 50 years of age.[3] This tumour was extensively studied in Japan because of the high prevalence in these areas. Interestingly it was found that the intestinal type of adenocarcinoma was predominant in high incidence areas whereas the diffuse type was more prevalent in in low incidence areas [4] Similarly, cancers of the antrum and pylorus are more common in high-incidence geographical areas, whereas cancers of the proximal cardia occur more commonly in low-incidence areas.[4]

Majority of patients are, unfortunately, diagnosed with advanced disease. Surgical resection is the mainstay of treatment for early stage cancer. The survival rate of patients with advanced resectable gastric cancer remains poor. For patients with unresectable tumours, chemotherapy is the treatment of choice. Platinum compounds, anthracyclines, fluoropyrimidines, taxanes and irinotecan are some of the drugs used in the treatment of gastric cancer.[1,4]

Helicobacter pylori infection is the single most common cause of gastric carcinoma.[5] Persistent infection and the consequent sequence of atrophic gastritis, intestinal metaplasia and dysplasia have been identified as a forerunner of gastric carcinoma. Other aetiological factors include tobacco smoking, high intake of salt-preserved and smoked foods, alcohol and bile reflux after gastric surgery.

Work on enzyme histochemistry in gastric carcinoma began as early as 1959 when aminopeptidase activity was discovered in the tumour. Similarly, the presence of alkaline phosphatase, and later acid phosphatase was detected in gastric carcinoma but there was no uniformity or specificity in their expression in the various histologic types.

Studies on molecular pathogenesis of gastric carcinomas were pioneered in 1992 by P. Wright et al. They found that they were prognostic differences in the expression of p53 and c-erb B2 amplification / over-expression between intestinal type and diffuse type of carcinomas and between early and advanced cancers. Numerous simultaneous studies have concluded that overexpression of p53 and c-erb B2 were more frequent in early intestinal than in early diffuse gastric carcinoma. Overexpression of c-met was greater in diffuse gastric carcinoma, especially in the advanced stages.

Loss of heterozygosity of Adenomatous Polyposis Coli (APC) gene and loss of heterozygosity of Deleted in Colorectal Carcinoma (DCC) gene were more common in the intestinal type of gastric carcinoma. Mario Scartozzi et al in 2004 studied about DNA copy number changes, Micro-Satellite Instability (MSI), thymidylate synthase, E-cadherin, beta-catenin, mucin antigen, p53, c-erb B2, COX-2, matrix metalloproteinases, VEGFR and EGFR.[6]

The main immunohistochemical markers for gastric carcinomas are Cytokeratin (CK). Mucin (MUC), Epithelial Membrane Antigen (EMA), Carcino-Embryonic Antigen (CEA) etc [6]. Many studies are being done and still continuing in this field. Researchers are giving stress to the prognostic significance of these markers and their role in determining the treatment modalities. One such upcoming marker is HER2. Recent literature illustrates HER2 as a molecular biomarker, which shows amplification and overexpression in gastric carcinoma and is associated with a poor prognosis.[7,8]

Various studies have shown that HER2 has a role in the development of many human cancers including gastric cancer (7 - 34 %). HER2 / neu (Human Epidermal Growth Factor Receptor 2) protein is a member of the Epidermal Growth Factor Receptor (EGFR) family. It is a plasma membrane bound Tyrosine Kinase (TK) receptor. It activates MAPK, P13K / AKT, PKC, STAT pathways. Signaling through these receptors promote cell proliferation and opposes apoptosis. HER2 gene is located on chromosome 17q21. Amplification of the gene products in cellular membrane cause acquisition of advantageous properties for malignant cells.[7,8]

Demonstration of HER2 overexpression in gastric cancer using Immunohistochemistry (IHC) was first described in 1986. FISH was used by some workers for the demonstration of HER2 overexpression.[5] HER2 plays a major role as a prognostic factor in gastric cancer. It is considered as a poor prognostic variable, second only to nodal metastases. HER2 staining intensity has been correlated with tumour sites within the stomach, serosal invasion and lymph node metastases. HER2 amplification is associated more with the intestinal histologic type. It is more commonly expressed by tumours arising from the gastro oesophageal junction than tumours elsewhere in the stomach. HER2 expression not only serves as a prognostic indicator but it also forms the basis for identification of patients who could benefit from targeted monoclonal antibody therapy with trastuzumab. A better understanding of the molecular basis of cancer has contributed to the development of targeted therapy.[9,6]

Numerous researchers have pioneered work on this marker with majority of the workers being Japanese scientists. A study done by Toshihiro Ishikawa et al in 1992 showed HER2 overexpression in 19 out of the 105 cases (18 %). P. Jung et al in 2006 demonstrated HER2 amplification in 3.8 % of gastric cancers. They went a step further and added that intestinal type cancers exhibited a higher rate of HER2 / neu amplification than the diffuse type cancers. According to them, age, TNM stage and amplification of HER2 / neu were all independently related to survival.[5]

Another study by Juntilla et al in 2004 showed HER2 / neu amplification in 12.2 % of gastric and 24 % gastroesophageal adenocarcinomas, and is more common in intestinal type than diffuse type of gastric carcinoma.[10] They also noted co-amplification of topoisomerase II alpha in majority of the gastric cancer. They stated that HER2 amplification was associated with a bad prognosis. C. Gravalos et al in 2008 conducted a similar study, in which

they found that HER2 positivity is associated more with intestinal type gastric carcinoma and carcinomas of the gastroesophageal junction.[11] In the same year, Hoffman et al tried to establish a HER2 scoring system for gastric carcinoma.[8] In their study, HER2 overexpression was detected in 10.7 % of gastric cancer samples, in which 83.3 % of tumours were intestinal type and 16.7 % were diffuse type. Concordance between IHC and FISH was 93.5 %.

S.D. Xie et al in 2009 did a study to assess HER2 expression in gastric carcinomas and to know its prognostic significance; in their study 18.8 % of gastric cancer specimens showed positive HER2 expression and no relationship was found between HER2 expression and clinicopathological parameters.[6] However, they concluded that HER2 expression was a poor prognostic indicator. Andreas H. Marx et al in 2008 published a study in which 16 % of gastric carcinomas were positive for HER2 and topoisomerase II alpha amplification. They used both immunohistochemistry and FISH.

Similar studies were also conducted in our country. In 2012, A. Sekaran et al noted overexpression of HER2 in 44.2% of gastric adenocarcinomas with prevalence of 34 % in intestinal type and 6 % and 20 % of diffuse and mixed types respectively [1]. A study conducted by Aditi R et al in 2015, in a tertiary care centre in South India noted HER2 overexpression in 27.6 % of gastric carcinoma, of which 93.8 % cases were of intestinal type.[12] The study also showed no significant relationship to age, sex, tumour site, tumour differentiation and stage.

With this background, the present study was undertaken:

1. To assess the expression of HER2 in gastric carcinoma specimens.
2. To determine if there is any relationship between HER2 expression with age and sex of the patient, site of tumour within the stomach, histologic type, tumour differentiation and stage of the disease.

METHODS

This cross-sectional study included all gastrectomy specimens which were received in the Department of Pathology, Government Medical College, Trivandrum and were diagnosed as gastric carcinoma, during the study period of two year from January 1, 2017 to December 31, 2019. The study was undertaken only after obtaining the institutional ethics clearance. A total of thirty-eight cases were included in this study. The sample size was calculated using the formula:

$$n = \frac{(Z_{1-\alpha/2})^2 PQ}{D2}$$

P is the proportion of HER2 expression in gastric carcinoma as per reference. P=27 %, Q=100 – P=73
Absolute precision =15 %, Confidence interval =95 %

$$\text{Sample Size} = \frac{3.84 \text{ x } 27 \text{ x } 73}{15 \text{ x } 15} = 34$$

The gastrectomy specimens were fixed in formalin, paraffin embedded and thin sections of 5 microns were prepared. The slides were stained using haematoxylin and eosin. The paraffin blocks of those slides with tumour were collected. Unstained thinner sections of 4 microns were prepared from the same on APES (Amino-Propyltri-Ethoxy-Silane) coated slides and incubated overnight for adequate fixation. The slides were deparaffinised and dehydrated. Endogenous enzymes were blocked and antigen retrieval was done. The primary antibody, HER2, was added, followed by the addition of the secondary antibody and chromogen. Sections were counterstained and mounted. HER2 expression in all the 38 cases were studied. HER2 is expressed on the cell membrane and membrane positivity with 2 + and 3 + scores were taken as positive expression.[1,8,12] IHC scoring of HER2 expression in gastric adenocarcinoma in biopsy and resected specimens is as follows:

Type of Specimen	Reactivity Characteristics	Score / Classification
Resection Specimens	No reactivity at 40X magnification Membranous reactivity in < 10 % of cells	0 / Negative
	Faint / Barely perceptible partial membranous reactivity in >10 % of tumour cells (at 40X magnification)	1 + / Negative
	Weak / Moderate complete OR basolateral / lateral membranous reactivity in > 10 % of tumour cells (at 10X magnification	2 + / Equivocal
	Moderate / Strong complete OR basolateral / lateral membranous reactivity in > 10 % of tumour cells OR unequivocal membranous staining at low magnification (at 4X magnification)	3 + / Positive
Small Biopsy Specimens	Any single cluster of tumour cells (> / = 5 cells) demonstrating IHC 3+ staining characteristics	3 + / Positive

Table 1. Method of IHC Scoring

Statistical Analysis

The data is entered in an Excel sheet with all the relevant details and analysis of the data was done using SPSS version 23.0. Pearson chi-Square test was done to establish if any correlation existed between HER2 expression and clinicopathological characteristics like age, sex, tumour site, histopathological type, grade of tumour and stage at diagnosis.

RESULTS

The types of specimens included total gastrectomy, subtotal gastrectomy, oesophagogastrectomy, proximal and distal gastrectomy. Of the total 38 specimens, 12 were total gastrectomy, 9 subtotal gastrectomy, 8 distal gastrectomy, 6 oesophagogastrectomy and 3 proximal gastrectomy. Gastric biopsies were excluded from the study.

The age varied from 30 years to 80 years with the majority of cases in the age group 50 – 70 years.

There was a definite male preponderance (71 %). The most common location of the tumour was in the pyloric region (42 %), followed by the fundus (29 %), gastro-oesophageal junction (21 %) and body (8 %). The most common gross type of gastric carcinoma was the ulcerative type (71 %), followed by the polypoidal (16 %), fungating (8 %) and the infiltrating type being the least common type (5 %). The most common histopathological type of gastric carcinoma encountered was the intestinal type of adenocarcinoma (68.42 %). The other histologic types encountered were diffuse type (18.42 %), mucinous adenocarcinoma (5.26 %), signet ring cell type (2.63 %), adenosquamous (2.63 %) and squamous cell carcinoma (2.63 %). Out of the 26 cases of intestinal type of adenocarcinoma, the majority 16 were moderately differentiated, 6 cases were poorly differentiated, and the remaining 4 cases were well differentiated. Most of the cases were in stage II (45 %) and stage III (39 %) at the time of diagnosis. Among the 38 cases, only 3 cases each were in stage I and IV (8 % each).

HER2 expression in the 38 cases of gastric carcinoma were studied. Membrane positivity with 2 + and 3 + scores were taken as positive. Out of the total 38 cases, 10 cases showed positive HER2 expression. All the positive cases were histologically intestinal type adenocarcinoma. All other histological types were negative for HER2.

Histologic Type	Positive	Negative	% Positivity	% Negativity
Intestinal Type Adenocarcinoma	10	16	38.46 %	61.54 %
Diffuse Type Adenocarcinoma	0	7	0.00 %	100 %
Mucinous Adenocarcinoma	0	2	0.00 %	100 %
Signet Ring Cell Adenocarcinoma	0	1	0.00 %	100 %
Adenosquamous Carcinoma	0	1	0.00 %	100 %
Squamous Cell Carcinoma	0	1	0.00 %	100 %

Table 2. HER2 Expression in the Various Histologic Types

The relationship between HER2 expression and the clinicopathological characteristics were assessed: among 27 male patients, 6 were positive for HER2 and among the 11 female patients, 4 were positive. Out of 11 patients with age over 65 years, 2 were positive for HER2 and among 27 patients with age less than 65 years, 8 were positive. Considering the tumour location, HER2 expression is more in tumours located at the pyloric region (43.7 %), followed by tumours of the gastro-esophageal region.

HER2 expression was solely seen in the intestinal type of adenocarcinoma and the expression is associated with moderate and poorly differentiated tumours and tumours of Stage III (60 %). HER2 expression and histopathological type had a statistically significant relationship as the p value is < 0.05 (0.019). Similarly, the relationship between HER2 expression and stage of tumour was also statistically significant (0.002). However, the other four variables i.e., age, sex, tumour site and grade of the tumour had no statistically significant relationship with HER2 expression.

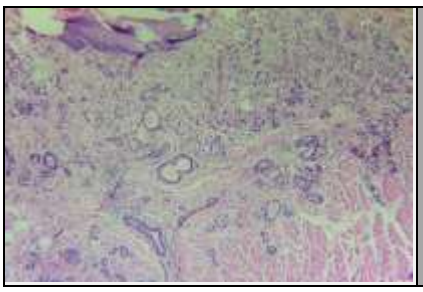

Figure 1. Intestinal Type Adenocarcinoma- Moderately Differentiated

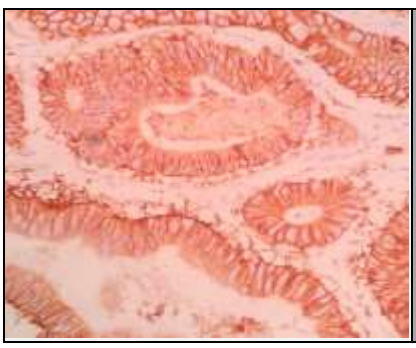

Figure 2. HER2 Strong 3 + Positivity in Intestinal Type Adenocarcinoma

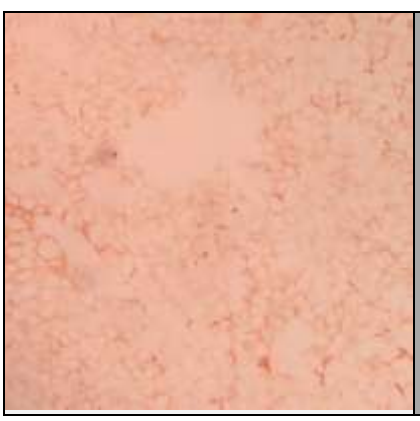

Figure 3. HER2 2 + Positivity in Intestinal Type Adenocarcinoma

Study Variable	Pearson Chi-Square Test	Significance (P Value - < 0.05)
Histopathological Type	0.019	Yes
Stage of Tumour	0.002	Yes
Age	0.467	No
Sex	0.369	No
Tumour Site	0.141	No
Tumour Grade	0.206	No

Table 3. Statistical Analysis of HER2 Expression & Study Variables

DISCUSSION

Carcinoma stomach is one of the most common malignancies worldwide and it is an aggressive disease. The upcoming treatment modality for advanced gastric carcinoma is targeted therapy using trastuzumab, which is a monoclonal antibody targeted against HER2 receptor.[11] Herein lies the significance of studying HER2 overexpression in gastric carcinoma which has been highlighted in many recent studies. Other prognostic variables include age, tumour stage, tumour location, its size and histologic type. Tumour stage is the most important prognostic determinant followed by the histologic type.

Most of the specimens were total gastrectomy specimens. Majority of the cases were in the age group 50 - 70 years and showed a definite male preponderance. The

mean age and sex distribution were on par with earlier studies. The most common location of the tumour was in the pyloric region. This was unlike other similar studies which showed antrum as the most common location followed by the pylorus.[12,13]

Histologically, most of the cases were of intestinal type of gastric carcinoma.[8,12,10,11] Most of the cases presented as Grade II (moderately differentiated) tumours. Majority of the patients who underwent surgery for the tumour presented in Stage III. The study of HER2 expression in gastric carcinoma was the primary objective of this study. HER2 positivity was noted in 26 % of cases, with positivity noted in intestinal type of adenocarcinoma (38.46 %). This is comparable with other similar studies where HER2 positivity ranged between 10 to 44 %.[1,8,12]

The secondary objective was to evaluate correlation between HER2 positivity and clinicopathological characteristics like age, sex, tumour site, histologic type, grade (differentiation) and stage. HER2 expression and histologic type and stage at diagnosis was statistically significant. However, HER2 expression had an insignificant relationship with age, sex, site of tumour and tumour differentiation. This is in concordance with other similar studies which also showed a similar significance.[8,12,10] At variance with other studies, this study showed a significant relationship with the stage of the disease.

CONCLUSIONS

HER2 overexpression was most commonly seen in intestinal type of gastric adenocarcinoma. The tumours were of a higher stage at the time of diagnosis. There was, however, no correlation with other variables like age, sex, tumour location and differentiation.

HER2 overexpression in gastric carcinoma is not routinely evaluated in our institution. This study could serve as a pilot study for alternate therapy with HER2 receptor antagonists like trastuzumab.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.
Our sincere gratitude to Dr. Krishna G., Professor and Head of the Department of Pathology for her valuable guidance and suggestions. We also thank Mr. Dileep Kumar P.D. and Mrs. Sheela S., Scientific Officers, who were an immense help with the IHC studies.

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