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Original Article

Evaluation of Pattern of HER2 neu Overexpression in Primary Gastric Carcinoma by Immunohistochemistry

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Abstract

Introduction: Gastric carcinoma is the fourth most frequent cancer worldwide, representing the second most common cause of death from cancer cases. Despite a steady decline in the incidence rate over the last few decades, the absolute incidence has risen due to the aging of the worldwide population. According to Lauren classification, it is divided into two major histological types, intestinal and diffuse. Very less is known about the molecular pathways induced by the HER2/neu receptor in gastric cancer compared to breast cancer. A descriptive study investigating the HER2/neu protein overexpression in gastric carcinoma was undertaken.

Materials and Methods: Our study was a descriptive study conducted for 2 years. Data for the study are obtained using patient diagnosis, case details, and examination of tissue sections. The sample size was calculated using odds ratio which comes out as 31. Cases were selected based on the inclusion and exclusion criteria. Permission was obtained from importer-exporter code before starting the study. Tissue samples of gastric carcinoma were obtained from gastrectomy and biopsy specimens which were analyzed using immunohistochemical staining. Statistical analysis is performed using SPSS 23 version.

Results: In our study, HER2/neu status was positive in 22.6% of all tested gastric cancer samples. Out of 31 cases, 7 cases show HER2/neu positivity of 3+, while 24 cases were HER2/neu negative or HER2/neu positive 1+ and none case showed HER2/neu positivity 2+ score. HER2/neu expression was a more common in intestinal type gastric cancer than the diffuse type (31.6% vs. 10%, respectively). HER2/neu positive 3+ cases are more common in those carcinoma located near gastroesophageal junction (57.1%) in comparison to carcinoma located in the body of the stomach (28.6%) and antral tumors (14.3%).

Conclusion: Gastric carcinoma occurring in this study population does not appear to differ considerably from that of their Western counterparts in terms of age of onset and sexual predilection. Further studies are required to prove more significant association with large sample size.

Key words: Gastric carcinoma, HER2/neu, Immunohistochemistry

INTRODUCTION

Gastric carcinoma accounts for 10% of cancers worldwide.[1] Despite a steady decline in the incidence rate over the last few decades, the absolute incidence has risen due to the aging of the worldwide population.[2] Cancers of the antropyloric region are more common in high-risk regions (Asia, Eastern Europe), whereas tumors of the cardia occur more commonly in low-risk regions (North America, Northern Europe).[3,4] Gastric carcinoma is the fourth most frequent cancer worldwide, representing the second most common cause of death from cancer cases (approximately 700,000/year).[5,6] Lauren divided gastric carcinoma into two major histological types, intestinal and diffuse types. Tumors that contain equal intestinal and diffuse components are defined as mixed tumors. Various etiological factors were associated with an increased risk of gastric cancer which includes chronic inflammation of gastric mucosa, exposure to diverse carcinogens, and genetic susceptibility, *Helicobacter pylori* infection, smoking and dietary habits (high intake of salt-preserved and/or smoked foods).

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HER2/neu (c-erbB2) is a proto-oncogene located on chromosome 17q21.[7] HER2/neu encodes a 185-kDa transmembrane tyrosine kinase receptor, a member of the epidermal growth factor receptor family (EGFRs), comprises four members: HER1 (EGFR), HER2, HER3, and HER4. They are involved in various aspects of tumor cell biology: Cell proliferation, apoptosis, adhesion, migration, and differentiation.[8] HER2/neu overexpression or amplification has been best studied in breast carcinoma but also reported in other solid tumors such as ovarian, endometrial, salivary gland, lung, esophageal, and gastric carcinomas.[9,10]

Various stromal-derived ligands, including EGF, EGF-like ligands, and neuregulins bind HER1, HER3, and HER4, inducing homodimerization and heterodimerization, phosphorylation of cytoplasmic tyrosine kinase moieties, and activation of complex signaling pathways essential for cell survival, differentiation, and proliferation.[11-14] HER2, however, is an orphan receptor with no known high-affinity ligand. HER2 becomes activated by heterodimerization after direct ligand binding by HER1, HER3, or HER4. Thus, the role of HER2 in the network of membrane receptor kinases seems to be as an amplifying coreceptor for HER1, HER3, and HER4.[12,13] A specific erbB-2 interacting protein (ERBIN) restricts the spatial distribution of the HER2 molecule to the basolateral membrane of epithelial cells.[15] ERBIN binds HER2, but not HER1, HER3, or HER4, and may be involved in connecting HER2 to cytosolic and cytoskeletal-associated components. Although much less is known about the molecular pathways induced by the HER2/neu receptor in gastric cancer compared to breast cancer, the current publication status suggests that the HER2/neu receptor plays a similar role in gastric cancer. There is evidence that HER2/neu is a prognostic factor for gastric cancer. Similar to breast cancer, HER2/neu over-expression correlates with a shorter overall survival.[13,16-18] For clinical HER2 determination, tissue-based methods, such as immunohistochemical (IHC) analysis and fluorescence *in situ* hybridization (FISH), have replaced whole-tissue extraction methods, such as Southern blot analysis, enzyme-linked immunosorbent assay, and polymerase chain reaction, which may require fresh tissue or suffer dilution owing to admixing of tumor and normal cells.[19] Frozen section IHC analysis, the "gold-standard" method for HER2 over-expression, is impractical in the current era of early cancer detection, in which tumor size often precludes ancillary testing of fresh tissue. IHC analysis is an attractive method for clinical HER2 determination owing to its retrospective potential and specific targeting of tumor cells. The plethora of available antibodies, methods, and grading schemes, however, has made standardization impossible.[20,21]

Up to now, fluoropyrimidine and platinum compound based therapies have been the standard of care. However, the prognosis of advanced gastric or gastro-esophageal cancer is poor with a 5-year survival rate of about 5-20%. HER2/neu overexpression was first described in 1986 using IHC.[13] Since then research is on, and it is turning out to be a useful molecule for which targeted therapy in the form of trastuzumab is available. This study is being undertaken to evaluate the pattern of HER2/neu protein overexpression in gastric carcinoma.

Aim and Objectives

Aim

To evaluate the pattern of HER2/neu expression in the primary gastric carcinoma cases by IHC techniques.

Objectives

1. To assess the pattern of HER2/neu positivity in cases of gastric carcinoma.
2. To determine the relationship between HER2/neu expression with clinicopathological parameters such as age, sex, grade, stage, and types of gastric carcinoma.
3. To observe any variation in HER2/neu overexpression in small biopsy and resected specimen obtained after NACT.

MATERIALS AND METHODS

This was a descriptive study conducted in a tertiary care hospital of an urban city between January 2013 and January 2015. Cases were selected from surgical pathology records in a retrospective as well as prospective way. The cases that had sufficient remaining tissue in the paraffin blocks (focus of interest for IHC stains) were identified and invited to participate in the study. A total of 31 cases were selected including small as well as total gastrectomy cases. The Institutional Ethics Committee approval was taken, and informed consent was received before beginning the study. Selected gastric carcinoma samples (paraffin blocks containing sufficient tissue left), hematoxylin and eosin (H and E) stained slides, and relevant data (age, treatment history available with requisition forms) have been collected. Standard IHC staining method using super sensitive polymer-horseradish peroxidase detection system (biotin-free) was used in the study. The additional benefit of using this method are:

1. Clean stain without endogenous biotin background
2. High signal to noise ratio for intense stain
3. Excellent sensitivity for weakly expressed antigens
4. Higher dilution of antibodies.

For each run of staining, a positive and negative control slide was also prepared. Prostatic carcinoma and benign prostatic tissue serve as positive and negative controls

that show the predicted staining patterns or the antigen under study. IHC analysis was performed as specified by the manufacturer using the Hercep Test kit. Briefly, 3 μm paraffin sections were placed in an oven overnight at 37°C. The slides were dewaxed in xylene, rehydrated in graded alcohol, incubated in citrate buffer at 95°C (pressure cooker). The slides then were placed on an immunostainer (DAKO) using the primary polyclonal antibody and polymer detection system supplied by DAKO. Following IHC analysis staining, the slides were placed in H for 1 min, dehydrated in graded alcohol, cleared in xylene, and coverslipped. Two pathologists independently scored slides as 0, 1+, 2+, or 3+ according to DAKO guidelines. Cytoplasmic staining was ignored. Only invasive tumor was scored. Scores of 0 or 1+ were regarded as immunohistochemically negative and 3+ as immunohistochemically positive. Discrepant IHC scores were resolved at the 2-headed microscope (Table 1).

RESULTS

The study included a total 31 cases. Further results are shown in Tables 2-4, Figures 1 and 2.

DISCUSSION

Gastric carcinoma remains an important problem among the Indian population. The male predominance observed in intestinal type of gastric carcinoma does not differ from what has also been previously noted by Lauren in a study in a large Finnish population. Currently, several molecular factors are studied as prognostic and predictive for gastric cancer. They include oncogenes and tumor suppressor genes, growth factors and receptors, cell adhesion molecules, proteolytic molecules and angiogenic factors (HER2, EGFR, p53, Cadherin,-catenin, cycloxygenase-2 [COX-2], matrix metalloproteinases, and vascular endothelial growth factor [VEGFR]).[22] Some of those prognostic factors can also be considered predictive for response to therapy as a molecular target to chemotherapeutics or a new class of antineoplastic molecules (HER2/neu targeted by Trastuzumab, COX-2 by nonsteroidal anti-inflammatory drugs, matrix metalloproteinases, EGFR and VEGFR by specific inhibitors).

A number of studies have analyzed HER2/neu overexpression in gastric cancer, and the rate of HER2 positivity is variable, ranging from 6% to 35%. Two major explanations for this discrepancy in HER2 expression are as follows: First, in the earlier studies polyclonal antibodies were used in contrast to more recent studies where monoclonal antibodies were used, and second, the more recent studies restricted their evaluation to membrane staining, excluding the cytoplasmic staining that was in the earlier studies considered in evaluation.[23] After the scoring system for gastric cancer was standardized by a panel of international oncology and pathology experts in 2007, the discrepancy in HER2 expression has become smaller.[24] The largest ongoing international trial which enrolled 2992 gastric or gastroesophageal junction carcinomas, defined 21.7% of evaluable tumor samples as HER2 positive.[25] In our study, HER2/neu status was determined by IHC, and all IHC 3+ score tumors are accepted as HER2/neu positive cases. In our study, out of 31 cases, 7 cases show HER2/neu positive 3+score, 24 cases were HER2/neu negative or HER2/neu positive 1+ score and no cases showed HER2/neu positive 2+ score. In this study, HER2/neu status was positive in 22.6% of all tested gastric cancer samples.

A high correlation between HER2 overexpression and intestinal type was reported by several authors in 1990s and confirmed in more recent studies. In the ToGA trial, HER2 positivity differed significantly by histological subtype (intestinal 34%, diffuse 6%, and other 20%). In this study, results are HER2/neu expression was more common in intestinal type gastric cancer than the diffuse type (31.6% vs. 10%, respectively). Adenosquamous type tumors showed no HER2/neu expression, probably due to small sample

Table 1: Reporting system of IHC

Staining intensity score	Surgical-staining pattern	Biopsies-staining pattern	HER2 Over-expression
0	No reactivity or membranous reactivity in<10% of tumor cells	No reactivity or membranous reactivity in any (or<5%) of tumor cells	Negative
1+(40×)	Faint/barely perceptible membranous reactivity in>10% of tumor cells; cells are reactivity only in part of their membrane	Tumor cell clusters with a faint/barely perceptable membranous reactivity irrespective of percentage of tumor cells stained (at least 5 tumor cells)	Negative
2+(10-20×)	Weak to moderate complete, basolateral or lateral membranous reactivity in>10% of tumor cells	Tumor cell clusters with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained (>5 tumor cells)	Equivocal (FISH Confirmation)
3+(2.5-5×)	Strong complete, basolateral or lateral membranous reactivity in>10% of the tumor cells	Tumor cell clusters with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained (>5 tumor cells)	Positive

IHC: Immunohistochemistry, FISH: Fluorescence *in situ* hybridization

Table 2: Age distribution of gastric carcinoma cases (*n*=31)

Type of gastric carcinoma	Age			
	Mean±SD	Median	Minimum	Maximum
Intestinal	61.42±14.366	65.00	27	79
Diffuse	55.90±12.635	57.00	39	77
Adenosquamous	65.00±0.000	65.00	65	65
Total	59.87±13.426	64.00	27	79

SD: Standard deviation

Table 3: Age distribution of gastric carcinoma cases according to site of carcinoma (*n*=31)

Location	Age			
	Mean±SD	Median	Minimum	Maximum
Antrum	57.79±17.777	65.00	27	79
Body	60.86±5.551	60.00	54	70
Fundus	62.10±10.525	64.00	44	75
Total	59.87±13.426	64.00	27	79
P value	0.735			

SD: Standard deviation

number. Different studies say the distribution of gastric carcinoma is increasing in the cardiac region,[26-29] but others have noted relatively stable time trends.[30-33]

In this study, it was found gastric carcinoma is more common in the antral region (45.2%) in respect to the body (22.6%) and fundus (32.3%). Irrespective of sex, the antral region is the most common site of gastric carcinoma (male - 45.5% and female - 44.4%). Mean age for gastric carcinoma located in the antral region is 57.79 years with a standard deviation (SD) of 17.77. The mean age for gastric carcinoma located in body is 60.86 years with an SD of 5.55 and for carcinoma located in the fundal region is 62.10 years with an SD of 10.52. HER2/neu positive cases with 3+ score are more common in those carcinoma located near gastroesophageal junction (57.1% in respect to carcinoma located in body of the stomach 28.6%, and antral tumors 14.3%. Different studies show that gastric carcinoma shows male predominance (8:1).[34] In this study, the incidence of gastric carcinoma is more common in male gender (male - 71%, female - 29%). As per Table 5 we have seen HER2 neu positivity ranging from 19.0% to 22.6% in different trials using different techniques like FISH or IHC

Maximum age of intestinal type of gastric carcinoma reported in our study is 79 years and minimum age is 27 years. The median age of intestinal type of gastric carcinoma is 65 years with a mean of 61.42 years and an SD of 14.366. Maximum age reported in diffuse type of gastric carcinoma is 77 years and minimum age is 39 years. The median age of diffuse type of gastric carcinoma is 57 years with a mean of 55.9 years and an SD of 12.63. Maximum

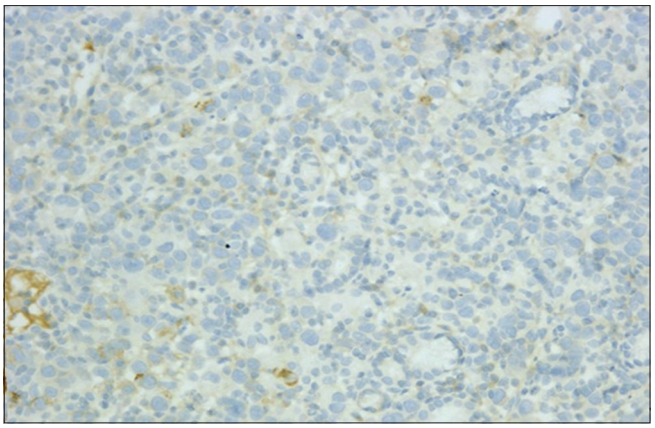

Figure 1: Immunohistochemistry slide showing HER2 neu positive staining score 1

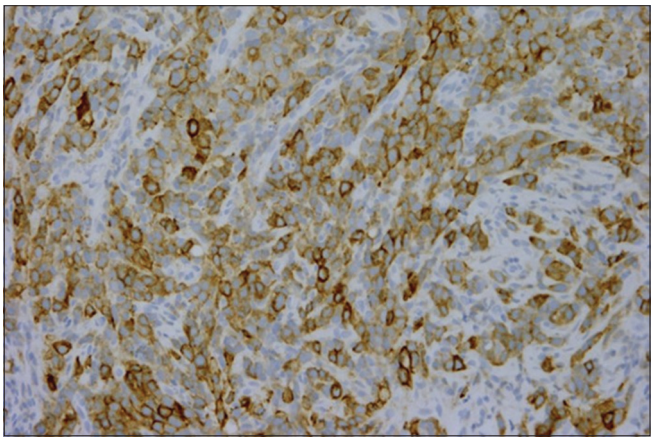

Figure 2: Immunohistochemistry slide showing HER2 neu positive staining score

age reported in the adenosquamous type of gastric carcinoma is 65 years and minimum age is 65 years. The median age of adenosquamous type of gastric carcinoma is 65 years with a mean of 65 years and a SD of 0.00.

CONCLUSION AND RECOMMENDATION

1. Before evaluation of the IHC slide, all morphologically atypical glands must be identified by H and E.
2. It is important to recognize that sensitivity of immunostaining may vary from laboratory to laboratory. Therefore, appropriate external and internal positive and negative controls must be used while interpreting the stain.
3. HER2/neu positivity must be evaluated with caution. A focal, weak, and noncircumferential staining patterns in benign-appearing glands should not be interpreted as indicative of a malignant diagnosis.
4. Finally, an internal ring study should be used as a good training method among pathologists who analyze HER2 expression in gastric cancer, especially because

Table 4: IHC of gastric carcinoma cases (*n*=31)

Variable	Type of gastric carcinoma			Total	*P* value	Significance
	Intestinal	Diffuse	Adenosquamous			
Gender						
Female	8 (42.1)	1 (10)	0 (0)	9 (29)	0.125	Not significant
Male	11 (57.9)	9 (90)	2 (100)	22 (71)		
Total	19 (100)	10 (100)	2 (100)	31 (100)		
HER2/neu1 Positivity						
Absent	6 (31.6)	1 (10)	0 (0)	7 (22.6)	0.306	Not significant
Present	13 (68.4)	9 (90)	2 (100)	24 (77.4)		
Total	19 (100)	10 (100)	2 (100)	31 (100)		
HER2/neu2 Positivity						
Absent	19 (100)	10 (100)	2 (100)	31 (100)	NA	NA
Present	0 (0)	0 (0)	0 (0)	0 (0)		
Total	19 (100)	10 (100)	2 (100)	31 (100)		
HER2/neu3 Positivity						
Absent	13 (68.4)	9 (90)	2 (100)	24 (77.4)	0.306	Not significant
Present	6 (31.6)	1 (10)	0 (0)	7 (22.6)		
Total	19 (100)	10 (100)	2 (100)	31 (100)		

IHC: Immunohistochemistry

Table 5: HER2 positivity rate in gastric cancer

Reference	Type of assay	*n*	HER2-positive rate (%)
ToGA trial	IHC/FISH	3667	22.1
Jørgensen[35]	IHC	6542	19.0
Jørgensen[35]	FISH/CISH	869	19.4
Present study	IHC	31	22.6

FISH: Fluorescence *in situ* hybridization, IHC: Immunohistochemistry

the scoring system for HER2/neu expression in gastric cancer differs from the one for the breast cancer. Due to the biological origin of gastric tissue, basolateral (not luminal) membranes are stained, resulting in incomplete immunoreaction of membranes of the tumor cells (typically "U" shaped). An identical scoring should be applied to samples with complete membranous reactivity, as well as those, where reactivity was restricted to the basolateral membrane if it was noted in 10%.

Limitations of the Study

1. The study is limited by its small sample size.
2. Follow-up of the cases was not feasible due to the wide residential spread of patients and lack of direct contact with laboratory post-surgery and hence was not included study design.
3. Unfortunately, the gold standard test (FISH) was not available for confirmation of HER2/neu overexpression.
4. Manual antigen retrieval technique, used in the present study, has its limitations compared to automated one.
5. In cases of very small endoscopic biopsy tissue, during antigen retrieval or subsequent treatment, loss of foci of interest was also faced frequently.

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