Indian Journal of Pathology and Oncology 2021;8(1):104–110

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pathology and Oncology

Journal homepage: www.ijpo.co.in

Original Research Article

Study of prognostic indicators and Her2neu expression in gastric adenocarcinomas – A tertiary care centre study

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ARTICLE INFO

Article history:
Received 13-11-2020
Accepted 17-11-2020
Available online 20-02-2021

Keywords:
Gastric adenocarcinoma
Immunohistochemistry HER2/Neu
Fluorescent in situ hybridization

ABSTRACT

Background: Gastric carcinomas rank 5th among all the cancers in India. The incidence increases with age and peaks during 5th to 7th decades. The most common location is gastric antrum followed by the lesser curvature. Her2 neu expression is reported as a frequent molecular abnormality in gastric adenocarcinomas associated with poor prognosis.

Objectives: The main aim was to study clinicopathological parameters of gastric carcinomas and study expression of HER2 in gastric adenocarcinomas.

Materials and Methods: This was a 4-year cross-sectional observational retrospective and prospective study of 47 cases of gastric carcinomas. The clinical profile and pathological features were studied. HER2 expression in gastric adenocarcinomas was scored according to Hoffman system and its association with age, gender, histopathological type, grade was studied. FISH was done in cases of equivocal HER2/Neu expression.

Results: Of the total 47 gastric carcinomas, it was most commonly found in antrum. Mean age of presentation was 59.4 years with male: female ratio of 1.4:1. Of total 47 adenocarcinomas, 22 were tubular, 4 cases each of papillary and mixed adenocarcinoma and 17 were poorly cohesive carcinoma. There were 5 cases of grade I, 27 grade II and 15 grade III carcinomas. HER2 positivity was seen in 10 cases, 5 were equivocal and 32 were HER2 negative. We found statistically significant correlation between HER2 expression with histopathological grade (P=0.003) and age (P=0.014).

Conclusions: Tubular adenocarcinoma was the common subtype and antrum was the commonest location. HER2 expression was significantly associated with grades and age in cases of gastric adenocarcinomas. This study helped us to know the trends of gastric carcinoma in our region. Use of immunohistochemistry and fluorescent in situ hybridization techniques additionally supports the use of targeted therapeutic modalities.

1. Introduction

Gastric carcinoma is the 5^{th} most frequently diagnosed cancer around the world.[1]

Gastric carcinoma tends to develop slowly over many years. Its incidence is known to increase with age and the peak incidence occurs at 5^{th} to 7^{th} decades.[2]

The average incidence of gastric carcinoma is 32.1 per 100,000 among males and 13.2 among females in Eastern Asia.[1]

A nationally representative survey in 2010 found that a total of 556400 deaths occur due to cancer in India and the mortality rate of stomach cancer is 12.6% in India.[3]

It has been reported that the survival rates were lower among smokers, alcohol drinkers, obesity and people who have the symptom of esophageal acid reflux and consume pickled, salty and smoked food.[1,3]

Prognostic factors such as age, gender, tumor location, morphology, lymphovascular invasion, lymph node metastasis, tumor stage, molecular profile, histological

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https://doi.org/10.18231/j.ijpo.2021.021

grade, perineural invasion, HER2/Neu expression and margins are important in determining the outcome of the disease and planning further management. The most powerful prognostic indicators in histopathology are depth of invasion, the extent of nodal and distant metastases.[4–6]

Understanding the association between prognostic factors and the survival rate for Gastric carcinoma is helpful to improve treatment efficacy.

HER2/Neu overexpression is a predictive of response to therapy.[7]

HER2/Neu is a protooncogene mapped on chromosome 17q12 and encodes transmembrane tyrosine kinase receptor (TKR) which comes under the epidermal growth factor receptor (EGFR) family whose phosphorylation initiates signaling pathways that lead to cell division, proliferation, differentiation, and apoptosis.[8]

HER2/neu status is primarily evaluated to determine patient eligibility for anti-HER2/neu therapy. Hence, inhibitors of HER2 membrane signals through anti-HER2/neu antibodies, that is trastuzumab (Herceptin) or lapatinib are associated with improved disease outcome in patients with primary or metastatic carcinomas.[9]

HER2/neu gene amplification and/or overexpression has been reported in up to 30% of breast cancers and in 9% to 38% of gastric carcinoma patients.[9] Overexpression in stomach carcinoma varies with differentiation (moderately differentiated greater than poorly differentiated) and histologic type (intestinal-type greater than diffuse type.[9,10]

This study was undertaken to evaluate prognostic factors of gastric carcinoma using WHO 2019 classification along with IHC expression of HER2/Neu in all cases of gastric adenocarcinomas.

2. Materials and Methods

This study included 47 cases of gastric carcinoma studied for a period of 4 years from 2016 to 2020. Patient's clinical details, histopathological examination (Using CAP protocol 2020), IHC for HER2/ Neu were studied.

In retrospective cases, paraffin-embedded blocks were retrieved from the departmental archives. Fresh slides will be prepared and stained with hematoxylin and eosin.

Immunohistochemistry (IHC) for HER2/Neu (C-erb-2 Oncoprotein, Dako A0485) was carried out and the results were recorded as positive, negative, and equivocal according to the staining patterns (ToGA trial using Hoffman et al 2008).[11]

Fluorescence in situ hybridization (FISH) was carried out in HER2/Neu equivocal cases. The slides were analyzed with a Leica DM2000 microscope equipped with an x1000 oil immersion objective, appropriate fluorescence filters, and Cyto Vision imaging software.

HER2 and CEP17 signals were counted in 20 cells after scanning each tissue core for an area with an increased number of HER2 signals.

Cases were scored using the American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) guidelines for HER2 testing in gastric cancer as follows: HER2/CEP17 ratio $\geq$2.0 was taken as positive, while HER2/CEP17 ratio <1.8 considered as negative; and HER2/CEP17 ratio between 1.8 to 2 as equivocal. If the ratio was between 1.8 to 2.2, an additional 20 nuclei were scored and the overall ratio calculated.[12,13]

The Kaplan-Meier method was used to calculate rates of overall survival.

3. Results

Among 47 cases included in the study, 43 were endoscopic biopsies cases (91.5%) and 4 were Major resections (8.5%).

The age of patients ranged from 21 to 81 years, with a mean age of 59.4 years (Table 1) with highest number of cases were seen in more than 50 years.

Table 1: Age group and gender wise incidence of gastric carcinomas

Age group in Years	Number of Males	Number of Females	Number of Cases n (%)
21-30	00	01	01 (2.1)
31-40	02	01	03 (6.4)
41-50	03	06	09 (19.2)
51-60	04	07	11 (23.4)
61-70	08	02	10 (21.3)
71-80	09	03	12 (25.5)
81-90	01	00	01 (2.1)
Total	27	20	47 (100)

Gastric adenocarcinoma were more common in males 27 (57.4%) compared to females 20 (42.6%) with M: F ratio of 1.4: 1. The most common clinical history was abdominal pain (29 cases), weight loss (20 cases), dysphagia (13 cases), vomiting (10 cases), anorexia (10 cases), ascites (10 cases), and abdominal lump (06 cases). Many of them have overlapping features.

The most common endoscopic findings were ulceroproliferative growth in 37 cases (78.7%) (Table 2)

Table 2: Endoscopic findings incidence of gastric carcinomas

Endoscopic Findings	Number of cases n (%)
Ulceroproliferative Growth	37(78.7)
Polypoidal Growth	02 (4.3)
Edematous Mucosa	02 (4.3)
Gastric Perforation	01 (2.1)
Nodular Growth	01 (2.1)
Gastric Outlet Obstruction	01 (2.1)
Mucosal thickening and Ulceration	01 (2.1)
Gastric Ulcer	02 (4.3)
Total	47 (100)

The most common site of the tumor was antrum 25 (53.2%) cases followed by lesser curvature in the body of stomach 07 (14.9%) cases and Gastroesophageal Junction (G-E Junction) 06 (12.7%) cases. (Table 3)

Table 3: Site distribution of gastric carcinoma

Site	Number of cases n (%)
G-E Junction	06 (12.7)
Cardia	02 (4.3)
Body (Lesser Curvature)	07(14.9)
Incisura	02 (4.3)
Antrum	25 (53.2)
Pylorus	05 (10.6)
Total cases	47 (100)

Among a total of 47 gastric carcinoma cases, 22(46.8%) were tubular adenocarcinoma, 4 (8.5%) cases each of papillary and mixed adenocarcinoma, and 17 (36.2%) were poorly cohesive carcinoma. Of these 17 poorly cohesive carcinoma cases, 10 (58.8%) cases were poorly cohesive signet ring cell carcinomas (Figure 1).

Out of 47 cases, there were 5 cases of grade 1 ((10.6%) 27 (57.4%) cases of grade 2 and 15(31.9%) cases of grade 3 carcinomas.

Of these 4 major resection specimens of gastric adenocarcinoma, lymph node dissection was done in all 4 cases of which 02 (50%) cases showed lymph node involvement by tumor. Of these 4 cases, 02 (25%) cases showing lymphovascular invasion.

HER2/Neu positive (3+) cases were seen in 10 (21.3%) cases followed by equivocal (2+) in 5 (10.6%) cases and negative (1+ or 0) in 32 (68.0%) cases. (Table 4)

Table 4: Incidence of HER2/Neu overexpression in Gastric adenocarcinomas

HER2/Neu overexpression	Number of cases n (%)
0 (Negative)	26 (55.3)
1+ (Negative)	06 (12.8)
2+ (Equivocal)	05 (10.6)
3+ (Positive)	10 (21.3)
Total	47 (100)

HER2/Neu status in 02 cases of which biopsies and surgical resection specimens were available. One case showed HER2/Neu 3+ positivity both in biopsy and surgical resection specimen and other cases showed HER2/Neu negativity (0) in biopsy and 1+ in surgical resection specimens suggesting evidence of HER2/Neu heterogeneity.

A statistically significant relation was found between HER2/Neu expression with age (p value =0.014) and histopathological grade (p value =0.003). (Tables 5 and 6)

There were no significant difference between HER2/Neu positive and negative patients in terms of gender, tumor site, histological type of the tumor, tumor extension, depth of invasion and metastatic site. (Table 5)

We did Fluorescence in situ hybridization (FISH) in 5 equivocal cases of immunohistochemistry. These equivocal cases of HER2/Neu showed HER2/CEP17 ratio < 1.8, that is not amplified or negative (ASCO/CAP guidelines for HER2) on FISH study.

Follow-up information was available in 23 cases which included 07 HER2- positive (3+) cases, 1 HER2 equivocal (2+) case, and 15 HER2 negative (1+/0) cases. The rest of the cases (24 cases) were lost to follow up.

The mean survival time for gastric carcinoma is 18.8 months. The median survival time for gastric carcinoma is 15 months.

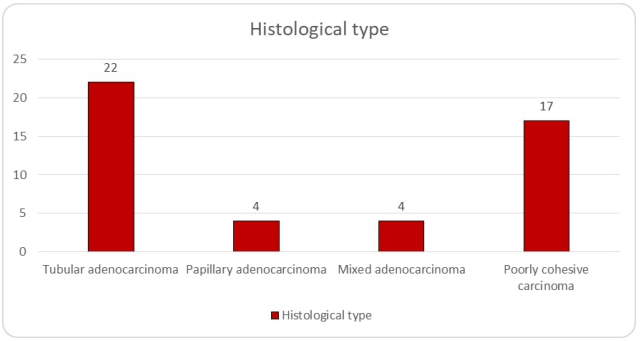

Fig. 1: WHO classification (2019): Histological type distribution of Gastric Carcinomas

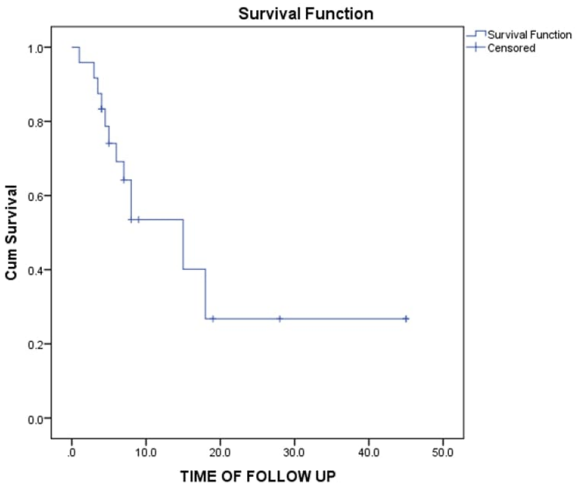

Fig. 2: Kaplan-Meier survival analysis of Gastric Carcinomas

4. Discussion

The peak incidence of gastric carcinoma is 50 to 70 years.[2]

In the present study, the highest number of cases were seen in more than 50 years of age that is 34 (72.3%) which coincides with the study by Nadaf AS et al (2018).[15] Most common age group in males was 61 to 80 years and in

Table 5: Univariate analysis of HER2/Neu overexpression and different clinicopathological features of the studied Gastric carcinomas

Clinicopathological features	HER2/Neu -ve cases (0/1+)	HER2/Neu equivocal cases (2+)	HER2/Neu +ve cases (3+)	Total	P value
Sex					
Male	19	4	4	27	0.311
Female	13	1	6	20	
Age					
≤ 50 years	8	4	1	13	0.014
>50 years	24	1	9	34	
Tumor site					
G-E Junction	6	0	0	6	0.405
Cardia	2	0	0	2	
Lesser curvature	4	0	3	7	
Incisura	2	0	0	2	
Antrum	14	5	6	25	
Pylorus	4	0	1	5	
Histological type					
Tubular adenocarcinoma	13	4	5	22	0.323
Papillary adenocarcinoma	3	0	1	4	
Mixed adenocarcinoma	4	0	0	4	
Poorly cohesive carcinoma	7	0	0	7	
Poorly cohesive signet ring cell carcinoma	5	1	4	10	
Histological grade					
G1	1	0	4	5	0.003
G2	20	5	2	27	
G3	11	0	4	15	
Tumor extension					
Lamina propria	12	3	3	18	0.118
Muscularis mucosae	20	2	4	26	
Peritoneum	0	0	1	1	
Serosa	0	0	1	1	
Subserosa	0	0	1	1	

Table 6: Comparison of HER2/Neupositivity and histological grade with other studies

	Laboissiere RS et al (2015)[14] n=124	Nadaf et al (2018)[15] n=70	Raj N et al (2018)[16] n=65	Present study n=47
Histological grade	HER2/Neu positivity	HER2/Neu positivity	HER2/Neu positivity	HER2/Neu positivity
Grade I	3.2%	26.4%	9.2%	8.5%
Grade II	5.6%	10%	18.5%	4.25%
Grade III	1.6%	23%	13.8%	8.5%

Fig. 3: Gross image ulceroproliferative growth in distal stomach

Fig. 4: Gross image of thickening of gastric wall

females was 51 to 60 years. Studies have shown that more than 90% of gastric cancer cases occur in people aged 50 or older (Gunderson LL, et al. 2014).[17]

The present study shows the most common histological type as tubular adenocarcinoma in 46.8% of cases which correlates with Abdel-Salam et al. (2018).[18] The second most common category was poorly cohesive carcinomas including signet ring cell type as 17 (36.2%) cases.

The present study showed HER2/Neu positivity of 21.3% correlating with Nadaf et al[15] which showed HER2/Neu positivity of 23.0% and Rajagopal et al.[19] showing HER2 positivity of 26.7%. We found statistical significance of HER2/Neu expression with histological grade (p value =0.003) and age (p value =0.014) which correlated with Raj

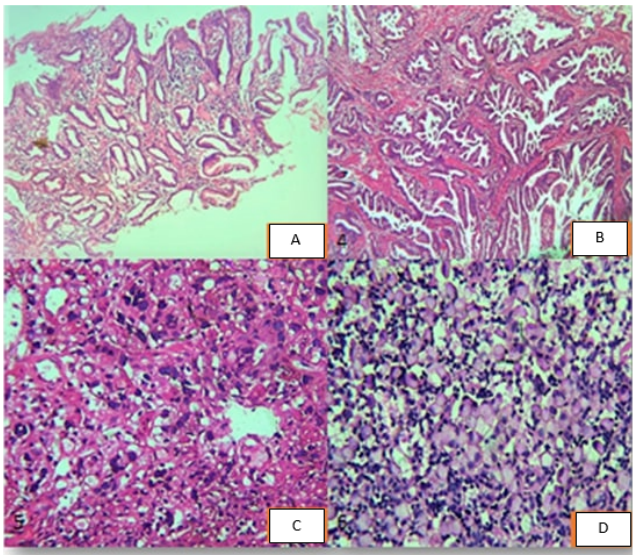

Fig. 5: A): Well differentiated Tubular adenocarcinoma (H&E 100x); **B):** Papillary adenocarcinoma with fibrovascular core (H&E 100x); **C):** Poorly cohesive carcinoma (H&E 400x); **D):** Poorly cohesive signet ring cell carcinoma (Periodic acid-Schiff stain, 400x)

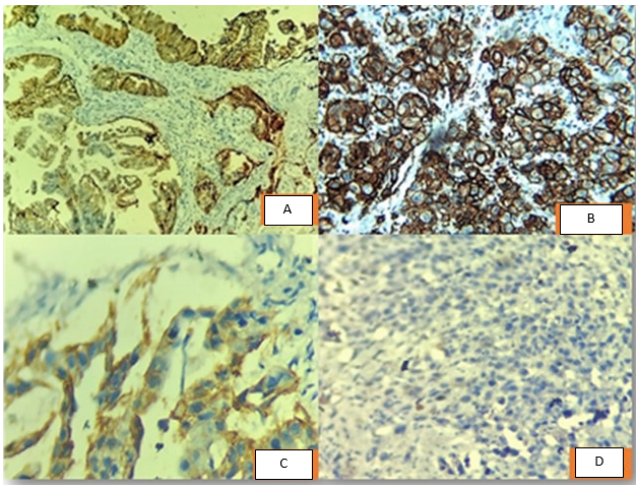

Fig. 6: A): Papillary adenocarcinoma, HER2/Neu 3 +; **B):** Poorly cohesive signet ring cell carcinoma, HER2/Neu 3+; **C):** HER2/Neu equivocal (2+); **D):** Her2/Neu negative (1+)

N et al.[16](Table 5)

The HER2/Neu overexpression was 66.7% in low-grade gastric adenocarcinoma as compared to 28.6% in higher grade in our study and showed statistical significance. Hence, low-grade gastric adenocarcinomas are more prone to show HER2/Neu positivity in comparison to high-grade tumors. Because of tumor heterogeneity, we found HER2/Neu positivity (3+) in a lower and higher grade of gastric adenocarcinomas.

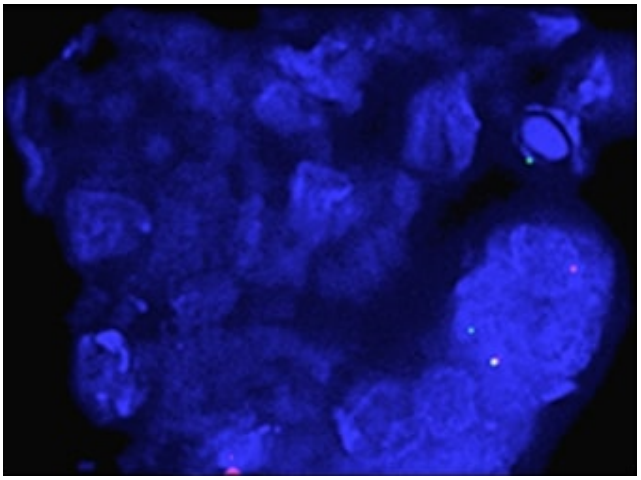

Fig. 7: FISH for HER2/Neu gene- without an amplification, green signals of probe showed HER2 gene region and orange signals showed CEP 17. (FISH, HER2 gene 1000x)

The differences between HER2/Neu expression in breast and gastric carcinomas are that in breast cancer, there is a predominantly circumferential membranous distribution of the antibody in the neoplastic cells, no intratumoral heterogeneity and variability in anatomical location; whereas in, gastric adenocarcinomas the staining is mostly incomplete, predominantly basolateral ("U"-shaped) or lateral (parallel lines), intratumoral heterogeneity, and incidence of HER2 expression in gastric cancer with anatomic location is variable. Gastric carcinomas are more frequent in the proximal stomach, esophageal-gastric junction than in the distal stomach.[20]

In our study, 5 cases with equivocal HER2/Neu showed HER2/CEP17 ratio < 1.8.

The study was done by Liu X et al (2016) in 122 equivocal cases showed HER2 gene amplification by FISH and compared the results with IHC in 122 equivocal gastric carcinoma cases, in which 17 out of 122 gastric carcinomas showed HER2/Neu amplification. The concordance rate between IHC and FISH was 13.9%.[21]

Satoshi Matsusaka et al. showed positive FISH in 47.3% of IHC score 2+ cases (61 of 129 patients) and 97.5% of IHC score 3+ cases (158 of 162 patients).[22]

He C et al. in year (2013) studied 197 gastric cancer cases in which 31 cases (15.74%) were identified as HER2 gene amplified by FISH, and 19 cases (9.64%) were scored as strongly positive for HER2 IHC staining (3+). The concordance rate between IHC and FISH analysis was 88.83%.[23]

Due to the heterogeneity of tumors in gastric adenocarcinoma, the expression of HER2/Neu is variable and cannot be used as a sole criterion for targeted therapy similar to breast carcinoma cases.

Hence, we recommended to do an HER2/Neu study to be done preferably on resection specimens rather than biopsy slides and needs more sampling of tumor tissue for better evaluation.

Our study provides an insight to the various prognostic factors including HER2/Neu expression for targeted and personalized therapy in gastric adenocarcinomas.

5. Source of Funding

None.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

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Cite this article: Patel BN, Lakhe R, Swami RM, Nimbargi R. Study of prognostic indicators and Her2neu expression in gastric adenocarcinomas – A tertiary care centre study. *Indian J Pathol Oncol* 2021;8(1):104-110.