

Clinicopathological Implication of HER2/neu Expression in Carcinoma Stomach

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Abstract

Background and Objectives Metastatic gastric carcinoma makes the current treatment options unsatisfactory. There is HER2 directed therapy like trastuzumab are used in metastatic gastric cancer. This study aimed to determine the tissue HER2/neu status by immunohistochemistry (IHC) and find their relation with clinicopathological parameters for assessing the prognosis.

Study Design, Materials, and Methods In this descriptive study, 30 patients of gastric cancer with metastasis were studied for HER2/neu expression by IHC. The chi-square test was used to determine the association between HER2/neu expression and clinicopathological parameters of patients.

Results and Conclusion The majority of samples (90%) were endoscopic biopsies from patients with a mean age of 61.4 years. Tumors were majorly of intestinal type (67%) with moderately differentiated grade located at the fundus of the stomach. The common site of metastasis was the lymph node (53.33%) followed by lymph node and liver metastasis (13.33%). The overexpression of HER2/neu by IHC was observed in 13.3% of cases. Furthermore, no significant association was observed between HER2/neu and gender, diet, age, tumor site, subtype, and grade. Overall, HER2/neu overexpression can be considered an independent prognostic factor for carcinoma stomach. Therefore, the patients identified with HER2/neu overexpression are targeted for trastuzumab therapy.

Keywords

- ▶ gastric carcinoma
- ▶ HER2/neu
- ▶ immuno-histochemistry
- ▶ metastasis
- ▶ trastuzumab

Introduction

Gastric cancer is one of the most significant causes of cancer-related morbidity and mortality worldwide. It is the fifth most frequently diagnosed and third leading cause of cancer-related deaths.¹ Most of the patients present with gastric cancer at an advanced stage increasing the frequency of metastasis, making the existing therapeutic options unsatisfactory. The median survival associated with this neoplasm

in advanced cases is only a year.² Hence, there is a need to identify molecular biology associated with the oncogenesis of gastric cancer and develop targeted therapies designed on a molecular level. However, there is increasing evidence that human epidermal growth factor receptor (HER2/neu) overexpression is a frequent molecular abnormality in gastric and gastroesophageal junction cancer. Taking clues from breast cancer treatment, trastuzumab (Herceptin) has been considered a potential treatment option in HER2/neu

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positive cases of gastric cancer. Immunohistochemistry (IHC) is one of the current standard methods for tissue HER2/neu status in gastric cancer. The frequency of HER2/neu over-expression in gastric and gastroesophageal junction cancer varies from 4.4 to 53.4% with a mean of 17.9%.³ There is a lack of Indian studies, hence a need to determine the frequency of HER2/neu in the Indian population for better patient management. Therefore, we aimed to evaluate HER2/neu expression status by IHC in gastric cancer. Furthermore, we also find the association of HER2/neu expression with different clinicopathologic parameters to identify the positive cases that may benefit from targeted therapy.

Materials and Methods

Study Population and Clinical Data Collection

This single-center descriptive study consists of consecutively sampled 30 patients having gastric cancer with metastasis on biopsies. However, the patients diagnosed with gastric cancer without metastasis and who were unwilling to give consent were excluded from this study. After obtaining informed consent from the patient, an endoscopic needle biopsy was taken from the primary, and in recurrent cases, from the metastatic site. Along with samples for routine histopathology examination, IHC was performed using the same samples. In addition, the patient's data regarding demography including diet history, radiological investigations (ultrasonography, computed tomography scan, magnetic resonance imaging), endoscopic findings, pathological score, and the clinical diagnosis details were retrieved from the patient's data sheet and the data was anonymized. The study data were compiled following the Declaration of Helsinki and was approved by the institutional ethics committee (# INST.EC/EC/096/2019-20, dated September 30, 2019).

Immunohistochemistry

According to the standard operating protocols of histopathological diagnosis at the hospital laboratory, the tissue biopsy is processed for paraffin-embedded block preparation. Briefly, 4 µm sections were mounted on poly-L-lysine-coated slides and incubated for 30 minutes at 60°C to deparaffinization. After rehydration in graded alcohol and water, a heat-induced epitope retrieval (HIER, pH 9) was performed for 10 to 30 minutes, followed by peroxide blocking for 5 minutes at room temperature. The tissue sections were incubated with 7 mL of prediluted HER2/neu primary monoclonal antibody (Clone# IHC042-7, GenomeMe, Canada) for 10 to 30 minutes at room temperature, and the subsequent steps were performed according to the kit protocols. The evaluation of HER2/neu expression scoring as defined by Hofmann et al⁴ was blinded for the data collection by the interpreter. The IHC score "0 or negative" means no reactivity or membranous reactivity in <10% of tumor cells, "1+ or negative" means faint or barely perceptible reactivity in ≥ 10% of tumor cells, and also cells are reactive only in part of their membrane, "2+ or equivocal" means weak to moderate, complete, basolateral, or lateral membranous reactivity in ≥ 10% of tumor cells, and "3+ or positive"

means strong, complete basolateral, or lateral membranous reactivity in ≥ 10% of tumor cells.

Statistical Analysis

The data collected were tabulated in MS Excel and analyzed using the IBM-SPSS software version 27.0. The association was tested using the chi-square statistical test.

Results

Demographics and Clinicopathologic Characteristics

The basic demographic and clinicopathologic characteristics of the 30 patients having gastric cancer with metastasis in this descriptive study are summarized in ►Table 1. The mean age of the patients was 61.1 ± 10.4 years, with the most common age of presentation ranging from 61 to 70 years (36.66%), which accounted for the largest group, followed by those within the age range of 51 to 60 (30%). Most of the metastatic gastric cancer patients were males (70%) and were nonvegetarian (56.67%) by diet, who were both fish and other meat eaters. For patients with metastasis, palliative care is preferred. Therefore, endoscopic biopsies (90%)

Table 1 Demographic and clinicopathologic characteristics of subjects (n = 30)

Characteristic	N, %
Age (y)	
< 50	05, 16.67
51–60	09, 30.00
61–70	11, 36.66
> 70	05, 16.67
Gender	
Male	21, 70.00
Female	09, 30.00
Diet type	
Nonvegetarian	17, 56.67
Vegetarian	13, 43.33
Specimen type	
Biopsy	27, 90.00
Resection	03, 10.00
Distribution of metastasis	
Bone metastasis	02, 06.67
Liver metastasis	03, 10.00
Lymph node metastasis	16, 53.33
Lymph node and liver metastasis	04, 13.33
Peritoneal metastasis	02, 06.67
Omental metastasis	02, 06.67
Malignant diffusion	01, 03.33
Tumor site	
Proximal	18, 60.00
Distal	12, 40.00
Histological type	
Diffuse	08, 26.67
Intestinal	20, 66.66
Mixed	02, 06.67
Grade of carcinoma	
Poorly differentiated	11, 36.67
Moderately differentiated	16, 53.33
Well-differentiated	03, 10.00

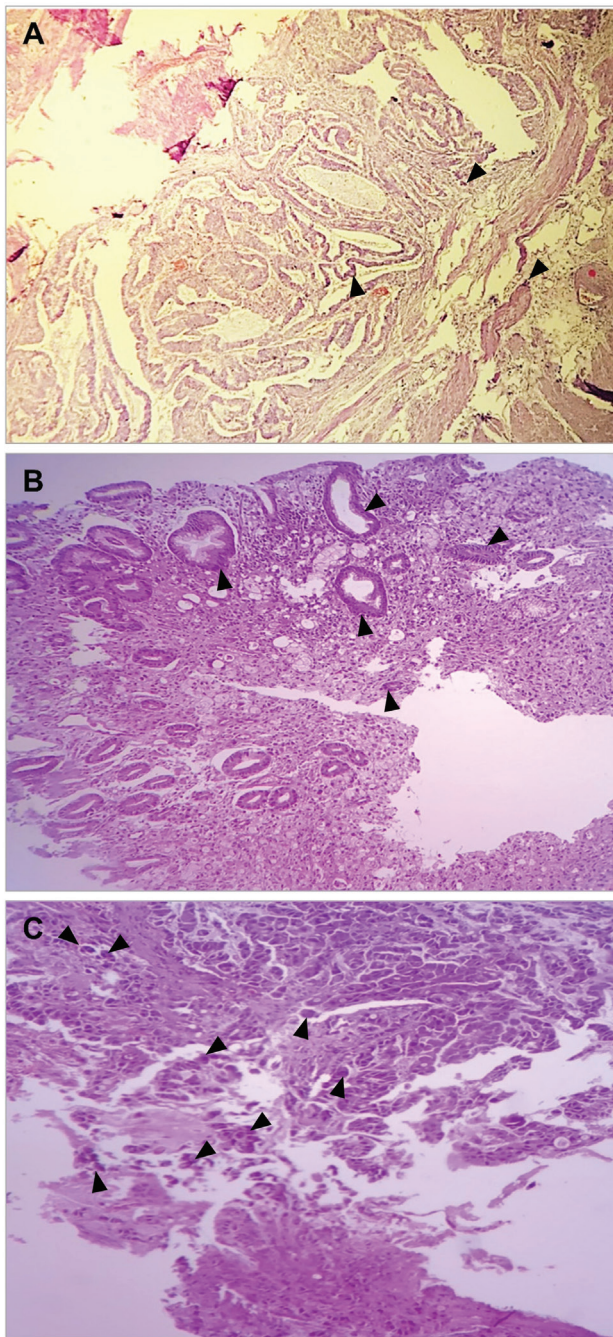


Fig. 1 Histopathological grading analysis showing well-differentiated (A), moderately differentiated (B), and poorly differentiated (C) adenocarcinoma of stomach (hematoxylin and eosin [H&E], 10 ×).

were taken in the majority of the study. Amidst gastrectomy cases, only three fulfilled our criteria of gastric cancer with metastasis (locoregional /distant) since their TNM staging was T4N1M1 with tumor deposits in the omentum. Lymph node metastasis was found to be the most common (53.33%), followed by both lymph node and liver metastasis (13.33%) and liver metastasis alone (10%). The proximal (60%) site of the tumor, with a highly intestinal (66.66%) histological type of distribution was the most common in the study subjects. However, the most common grade of gastric carcinoma was moderately differentiated (53.33%), followed by poorly differentiated (36.67%) tumors (► **Fig. 1**). Furthermore, the

Table 2 Immunohistochemical analysis of HER2 status in carcinoma stomach

HER2 status	Frequency (n)	Percent (%)
IHC score (n = 30)		
+0 (negative)	16	53.33
+1 (negative)	10	33.33
+2 (equivocal)	3	10.00
+3 (positive)	1	3.33

Abbreviation: IHC, immunohistochemistry.

chi-square test analysis showed that there was no statistically significant association of HER2/neu expression with age, gender, diet, tumor site, subtype, and grade.

HER2/neu Status in Carcinoma of the Stomach

The immunohistochemical analysis of 30 cases of carcinoma stomach for HER2/neu was positive in only 1 (3.33%) case, equivocal in 3 (10%) cases, while the remaining 26 were negative (86.67%) (► **Table 2**).

Discussion

The third most common cause of mortality worldwide due to cancer is carcinoma stomach.⁵ According to the National Cancer Registry, 32,713 patients with gastric cancer were identified in the year 2020, which is the sixth most common type of cancer in females, and the fifth most common type in Indian males.^{6,7} Therefore, it is important to determine and identify the gastric cancer histological type, grade, and HER2/neu expression. This would help to find patients who can benefit from monoclonal antibody-based trastuzumab therapy. With this background, this study aimed to assess the HER2/neu expression in advanced gastric carcinoma by IHC. The mean age of the study population was 61.4 years with the range of the patient varying from 33 to 80 years with a predominance in males. This could be due to the higher exposure of males to certain risk factors such as tobacco, alcohol, inorganic dust, nitrosamines, etc.^{8,9}

This study observed that nonvegetarian diet-following patients had a majority succumbing to carcinoma stomach (56.7%), compared to the vegetarian diet-based study population. In addition, the most common pattern of metastasis noted in this study was lymph node metastasis (53.33%), followed by both lymph node and liver metastasis (13.33%), and our findings were in concordance with the study by Versteegen et al.¹⁰ Several studies including our study showed the presence of tumor in the proximal portion (predominantly fundus) of the stomach.^{11–13} Microscopically, 67% of the carcinomas in this study were intestinal type, and similar findings were observed in other studies.^{14–16} Moreover, moderately differentiated carcinoma was the predominant grade followed by poorly and well-differentiated carcinoma similar to the Gupta et al study.¹⁴ Furthermore, this study did not find any statistical significance in the association of HER2/neu expression with age, gender, diet, tumor site, subtype, and grade; it might be attributable to the relatively low sample size.

The immunohistochemical analysis of HER2/neu status was according to Hoffman's scoring criteria for determining the IHC score.⁴ This included strongly positive membranous staining as a 3+ score, while cytoplasmic and luminal staining were not included. This study showed 13.3% positivity of HER2/neu by IHC, which lies in the range (8.2–53.4%) reported by various international articles from Japan, Australia, etc.^{16,18–20} Studies done before Hoffman's scoring criteria were developed, showed higher positivity. The broad variations in the HER-2/neu overexpression were due to different scoring criteria and interobserver variability. Another reason is the use of several methodologies to detect overexpression. The different populations (genetic differences) studied by the various groups could also be held accountable. The genetic differences among the various populations studied by different groups could be a factor for variation in the wide range of positivity for HER2 by IHC. This study found only four patients with HER2/neu positive status, which could be because of following the strict protocols including methodology and Hoffman's scoring criteria. Studies done recently, following standard protocols and scoring criteria, have reported HER2/neu overexpression close to 15%. A study done by Shan et al done on 1,463 patients showed 9.8% positivity.^{17,21}

Conclusion

Overall, this study indicates that HER2/neu expression is not associated with any of the clinicopathological parameters. Therefore, overexpression of HER2/neu can be taken as an independent prognostic factor in patients with carcinoma stomach. Moreover, HER2 positivity by IHC would help to decide the therapy to be implemented and it will further help in improving the overall quality of life and survival of patients with carcinoma stomach.

Conflict of Interest

None declared.

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