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PROGNOSTIC SIGNIFICANCE OF MEMBRANE ASSOCIATED HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 IN GASTRIC ADENOCARCINOMAS

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ABSTRACT

Background: The difficulty in detecting gastric cancer at an early stage and the lack of effective therapy all contribute to the high mortality. Human epidermal growth factor receptor2 (HER2) one of the first molecular targeted drugs developed, is a transmembrane glycoprotein, involved in the signal transduction pathways leading to cell growth and differentiation. Clinical demand for HER2 testing is rapidly increasing after the recent introduction of trastuzumab (anti-HER2 antibody) for the treatment of patients with breast cancers. The objective of this study was to evaluate the prognostic significance of HER2 overexpression in gastric adenocarcinomas patients. **Methods:** We included 63 prospective patients with gastric adenocarcinomas diagnosed at Sanjay Gandhi Postgraduate Institute of Medical Sciences between 2015-2017. Expression of HER2 oncoprotein was evaluated by immunohistochemistry using HER2 antibody. HER2 expression was further correlated with clinicopathological parameters. **Results:** Out of 63 cases of gastric adenocarcinoma, there were 48(76.2%) males and 15(23.8%) females with a mean age of 53.9 yrs. Moderately differentiated adenocarcinoma was predominant 27(42.8%) followed by poorly differentiated 24(38%) and well differentiated type 12(19%). HER2 positivity was observed in 40% of

cases. The HER2 expression was evaluated as staining score of 0 in 15(24%) patients 1+ in 23(36.5%), 2+ in 16 (25.3%), and 3+ in 9(14.3%) patients. HER2 positivity was not significantly associated with gender, tumor site (antropyloric), histological subtypes, tumor differentiation and depth of infiltration (p>0.05). **Conclusion:** Standardized scoring criteria and accurate assessment of HER2 expression in gastric adenocarcinoma is of importance and may be helpful in the optimal selection of patients for trastuzumab (Herceptin) therapy.

KEYWORDS: Gastric adenocarcinomas, HER2/NEU (Human Epidermal Growth Factor Receptor 2), Immunohistochemistry (IHC), Haematoxylin and Eosin (H & E).

INTRODUCTION

Gastric cancer (GC) is still the second most common cause of cancer death worldwide,[1-3] although the incidence and mortality have fallen dramatically over the last 50 years in many regions.[4,5] The incidence of gastric cancer varies in different parts of the world with highest incidence rates documented in Eastern Asia, Eastern Europe, and South America, while North America and Africa show the lowest recorded rates.[6,7-9] Gastric adenocarcinoma, a leading cause of cancer death worldwide is the fourth most common cancer in males and females respectively.[2,10-11] Globally, gastric cancer accounts for 9,89,600 new cases and 7,38,000 deaths annually. The number of new gastric cancer patients is approximately 34000 each year with male to female ratio of 2:1 in India.[12] The incidence of gastric cancer is known to increase with age with the peak incidence occurring at 60-80 years. Patients having gastric cancer(GC) younger than 30 years are very rare.[13,4] With increasing understanding of the molecular biology of HER2, and the availability of genomics and proteomics analyses, it has now been recognized that HER2 is implicated in other severe forms of cancer, notably gastric cancer. New molecular targeted therapies are urgently needed which interfere with signaling cascades involve in cell differentiation and survival. The importance of HER2 testing is increasingly recognized after the recent approval of trastuzumab (anti-HER2 antibody) for the treatment of HER2 overexpressed breast cancer. Various studies indicate a role of HER2 in the development of many types of cancer, especially in invasive breast cancer in which overexpression of the protein in the cell membrane and/or gene amplification has been detected in 10–34%, correlating with worse prognosis and being a predictor of poor response to chemotherapy and hormonal treatments.[15,16]

HER2 is a proto-oncogene encoded by ERBB2 on chromosome 17q22 play a pivotal role in growth factor signal transduction pathways leading to cell survival, proliferation and

differentiation.[15,17-19] It consists of four plasma membrane-bound receptor tyrosine kinases that transmit extracellular signals to initiate cellular signaling pathways via mitogen-activated protein kinase, phosphoinositide 3-kinase, phospholipase C, protein kinase C, and signal transducer and activator of transcription.[16,20,21] The HER2 overexpression rate in gastric cancer varies widely in the literature between 7%-34%.[15,22-29] In gastric cancer the prognostic value of HER2 is less established.[30-33]

Prognostic value of HER2 is still controversial with some studies identifying it as a negative prognostic factor, some as a positive factor, and some others find no association.[15,32,34-45]

The present study focused on the immunohistochemical expression of HER2 in gastric adenocarcinoma and their correlation with known clinical and histopathological prognostic parameters.

MATERIALS AND METHODS

Study population

This was a prospective study. A total of 63 patients who had undergone either total, distal or partial gastrectomy for adenocarcinoma of stomach or gastro esophageal junction between 2015 to 2017 at Sanjay Gandhi postgraduate institute of medical sciences, Lucknow, India were enrolled in this study. Relevant clinical details were collected from patient's case record files and from hospital information system. None of the patients had undergone prior preoperative radiation, chemotherapy or targeted therapy.

Ethics statement

Study was approved by the Institutional Ethical Review Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences in Lucknow, India.

Histopathological study

All gastrectomy tissue specimens were fixed in 10% buffered formalin, no longer than 30 minutes after resection, and for a fixing time of 8–48 hours. Tissues were processed and embedded in paraffin. Tissue blocks were cut in 3-5µ thick sections, fixed for 2hrs at 60˚C. Deparaffinised sections were stained with haematoxylin and eosin. Histopathological diagnosis was made and adenocarcinomas were classified according to Lauren classification into intestinal, diffuse and mixed type. Data regarding demographics and pathological

information like age, sex type of diagnosis, tumor localization and treatment modalities (surgery, chemotherapy, radiotherapy) were retrieved from patient's files.

According to WHO grading system, adenocarcinoma was graded into well differentiated, moderately differentiated and poorly differentiated.

Immunohistochemistry

Paraffin blocks containing representative samples of the tumors were selected by reviewing H&E stained slides. Formalin-fixed paraffin-embedded samples were sectioned in 3-5 µ thick sections, taken on poly L lysine coated slides, fixed for overnight at 60ºC, and then deparaffinized in xylene and hydrated in a decreasing series of alcohol. Endogenous peroxidase was blocked with 3% hydrogen peroxidase. Antigen retrieval was performed in a microwave oven for 30 minutes at 98˚C with the slides immersed in TRIS-EDTA (pH 8.5). Immunohistochemical staining was performed using Rabbit monoclonal antibody for HER2 (SP3: Labvision; Thermo Fischer Scientific, Fremont, CA, United States, dilution1:100) followed by addition of diaminobenzidine (DAB).

The slides were stained with hematoxylin for counterstaining and then mounted using DPx. Positive control for HER2 was a known HER2 overexpressed case of infiltrating ductal carcinoma.

Evaluation of IHC results

Immunohistochemistry evaluation was performed according to the modified gastric cancer testing protocol, taking incomplete basolateral or only lateral staining.

Slides were scored by a pathologist following the scoring system of Hofmann et al.[23] and Ruschoff J et al.[22]

IHC Score	HER 2 Protein Overexpression	Staining pattern (Surgical specimen)
0	**Negative**	No reactivity or no membranous reactivity in <10%any tumor cell.
1+	**Negative**	Faint or barely perceptible membranous reactivity in ≥10% of tumor cells. Cells are reactive only in part of their membrane.
2+	**Equivocal**	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells.
3+	**Positive**	Strong complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells.

Statistical analysis

Demographic characteristics of patients were assessed by descriptive statistics. The overexpression rate in each HER2 group (HER2-positive and HER2-negative groups) was compared using Pearson's chi-square test and Fischer exact test. We considered statistically significant difference when *P* was < 0.05.

RESULTS

Clinicopathological variables

The mean age of the 63 patients with gastric adenocarcinoma was 53.9 years. There were 48(76%) male patients and 15 (24 %) female patients. Majority of tumor were located in antropyloric region 37(59%) followed by GE junction 10(16%), body and antrum 7(11.1%), body and fundus 5(8%) and antropyloric with body lesion 4(6.3%). Out of 63 patient's depth of invasion were present only in 58 case (92.1%). According to Lauren classification, 38(60.3%) patients had intestinal type, 20(31.6%) had diffuse type and only 5(8%) patients had mixed pattern of adenocarcinoma. In terms of histological subtype maximum number of tumors were moderately differentiated type 28(44.4%) followed by poorly differentiated type 22(35%) and well differentiated type adenocarcinoma13(20.6%). The clinicopathological variables of gastric adenocarcinoma are summarized in Table1.

HER2 Expression

Twenty-five patients (40%) out of 63 cases of gastric adenocarcinomas were positive for HER2 immunoreactivity and 38(60.3%) were negative for HER2 staining. Her2 immunohistochemical score of 0,1+,2+ and 3+ were found in 15(24%), 23(36.5%), 16(25.3%), 9(14.3%) respectively (Table2 & Fig.1,2,3). HER2 immunohistochemical expression was correlated with clinicopathological parameters such as gender, depth of invasion, histological types and with tumor differentiation. HER2 positivity was found more frequently in moderately differentiated tumors 11(17.4%) followed by poorly differentiated tumors 9(14.2%) and well differentiated tumors 6(9.52%) Fig. 3A&B. HER2 overexpression was more in intestinal type 13(21%) than diffuse type 9(14.2%) and mixed type 3(5%). Table3a &3b summarizes HER2 expression status by subgroups (Lauren classification and with histological subtypes).

Table 1: Correlation between HER2 expression status and clinicopathological parameters.

S.No.	Clinical Variables	N=63	HER2 IHC Score		p-value
			Positive	Negative	
1.	**Gender**				0.544
	Male	48	20	28	
	Female	15	5	10	
2.	**Age**				0.010
	≤55	35	9	26	
	>55	28	16	12	
5.	**Tumor site**				0.893
	Gastric	53	25	38	
	GE-Junction	10	3	7	
6.	**Lauren Classification**				0.434
	Intestinal	38	13	25	
	Diffuse	20	9	11	
	Mixed type	5	3	2	
7.	**Tumor Differentiation**				0.973
	Well	12	5	7	
	Moderately	27	12	15	
	Poorly	24	10	14	
8.	**Depth of Invasion**				0.327
	Yes	58	25	33	
	No	5	0	5	

Table 2: Frequency of Immunohistochemical expression of HER2 Protein.

IHC Score	n=63 (%)	HER2 Protein expression
3+	9(14.3)	Positive
2+	16(25.3)	Equivocal
1+	23(36.5)	Negative
0/-ve	15(24)	

Table 3a: Correlation of HER2 expression with Lauren Classification.

Laurens Classification	Total number of patients	HER2 IHC Score			
		0	**1+**	**2+**	**3+**
Intestinal type	38	7	18	8	5
Diffuse type	20	4	7	6	3
Mixed type	5	2	0	2	1
Total	63	13	25	16	9

Table 3b: Correlation of HER2 with Histological grade differentiation.

Degree of Differentiation	Total number of patients	HER2 IHC Score			
		0	**1+**	**2+**	**3+**
Well differentiated	12	4	3	3	2
Moderately differentiated	27	5	10	8	4
Poorly differentiated	24	5	9	8	2
Total	63	14	22	19	8

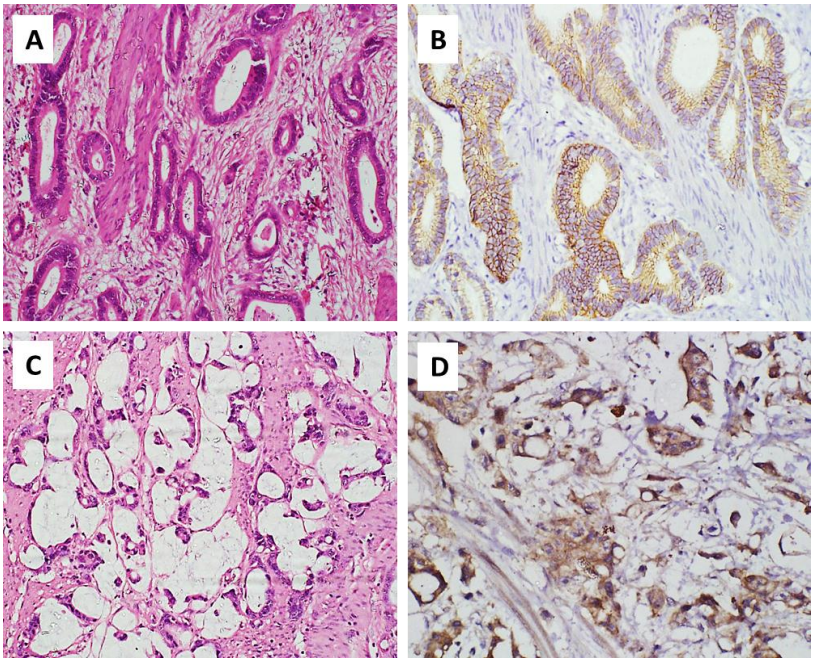

Figure 1: A-B: H&E of Moderately differentiated adenocarcinoma and corresponding IHC showing no HER2 staining on tumor cell (score-0, negative). B-C: H&E of Signet ring cell and corresponding IHC showing faint barely perceptible HER2 staining (score-1+, negative).

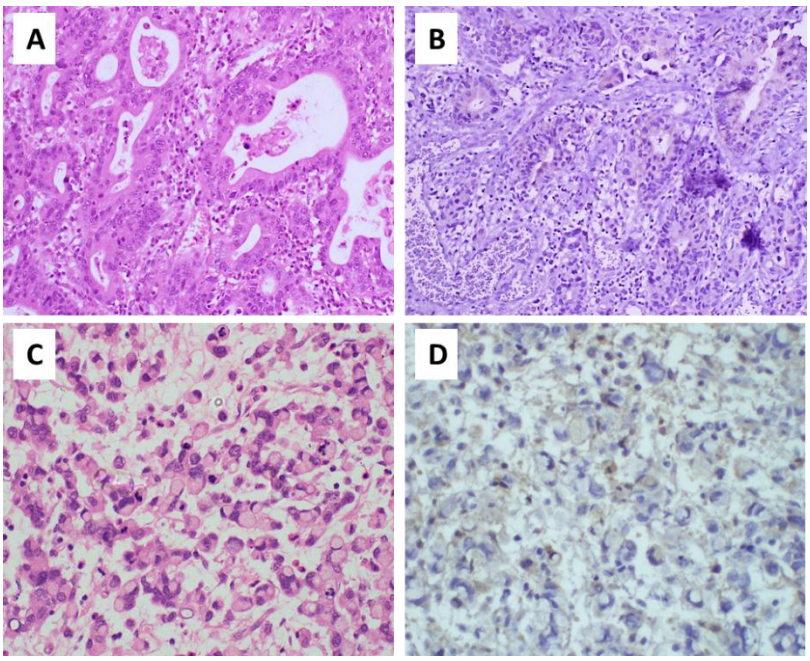

Figure 2: A-B: H&E of Moderately differentiated adenocarcinoma and corresponding IHC showing moderate complete membranous HER2 staining in glands (score-2+, equivocal). B-C: H&E of Mucinous adenocarcinoma and corresponding IHC showing HER2 immunostaining (score-2+, equivocal).

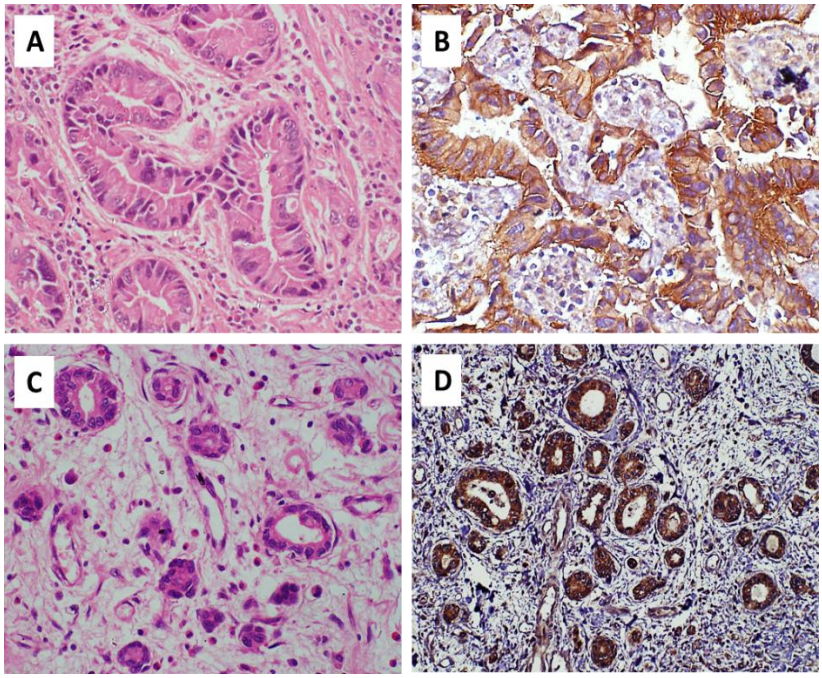

Figure 3: A-B: H&E of Moderately differentiated adenocarcinoma and corresponding IHC showing strong membranous HER2 staining in glands (score-3+, positive). B-C: H&E of well differentiated adenocarcinoma and corresponding IHC showing strong HER2 immunostaining (score-3+, positive).

DISCUSSION

Gastric adenocarcinoma is one of the most frequent carcinomas in the stomach. Although the incidence rate has shown a declining trend in recent years, the mortality rate is still quite high. The prognosis of gastric carcinoma depends mostly on the histopathological grade and the stage.[46,47] There is wide geographical variation in the incidence of gastric cancer within India, the highest incidence rate reported in North Eastern and South Indian states.[12] In certain parts of India, gastric cancer has been documented to be more common in south Indian males. The overexpression of HER2 in gastric cancer has been done only in few studies in India in which HER2 was seen in 44.2%, 9.6%, 21.4% and 26.6% cases.[48-51] In most of the studies HER2 expression was found in 7% to 34% in gastric adenocarcinomas cases.[15,23,24,26,27,52,53] In our study HER2 positivity was observed in 40% of cases. This differences might be due to differences in sample size, patient population, interpretation and scoring methods used in these studies.

Several studies have evaluated the relationship between HER2 status and prognosis in patients with gastric cancer.[24,27,32,34,36,38,39,54] Unlike in breast cancer, the studies in gastric cancer to date have yielded inconsistent findings regarding the prognostic relevance of

HER2. Some studies showed that HER2 positivity was associated with a significantly worse prognosis[24,27,32,34,39] whereas others found no association between HER2 status and prognosis.[36]

The results of HER2 overexpression in Indian patients' needs further validation by fluorescence insitu hybridization.

HER2 expression was more in intestinal type 13(20.6%) than diffuse type 9(14.2%) in our study as reported in other studies.[55-57] The association of HER2 with a specific histologic tumor type suggest that intestinal and diffuse type develop along different molecular pathway.

Similar to Rajagopal et.al,[48] for tumor differentiation the immunohistochemical staining pattern for HER2 was more in moderately differentiated adenocarcinoma 12(19%) followed by poorly differentiated 10(16%) and well differentiated adenocarcinoma 5(7.9%). In contrast to other study no relationship was observed between HER2 positivity with gender, Lauren classification, tumor differentiation and with depth of infiltration($p>0.05$) which is consistent with other study.[58]

In primary breast cancer it has been demonstrated that patients will only respond to Trastuzumab treatment if tumor show HER2 expression in cell membrane or HER2 gene amplification. Herceptin (trastuzumab) has been used for the treatment of HER2 overexpressing breast cancer for more than 10 years and was approved by the European Medicines Agency in 2010.So, far in GC patients HER2 expression has been reported in relatively small series, showing controversial results regarding its prognostic value. We didn't found any significant association between HER2 positive and negative patients in terms of gender, tumor location and depth of invasion. The discrepancies within these results is likely due to ethnicity, cancer histotype, use of different assays for HER2 evaluation and tumor heterogeneity.[59]

Intratumoral heterogeneity of HER2 status in gastric adenocarcinoma has also made the diagnosis of HER2 overexpression difficult and irreproducible.

CONCLUSION

HER2 as a prognostic factor in gastric cancer is still debate due to significant differences in study results, even though more recent studies favor its negative impact on prognosis. The

most important factor is likely a consequence of having no standardized HER2 test and scoring criteria. Further more research is required to explain the impact of HER2 on development and prognosis of gastric cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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