

# A study of the incidence and prognostic value of HER-2 overexpression in patients with gastric adenocarcinoma in Odisha

Sangram Keshari Panda<sup>1\*</sup>, Amita Panda<sup>2</sup>, Mishra JN<sup>1</sup> and Pallavi Bhuyan<sup>3</sup>

<sup>1</sup>PG Resident, Department of General Surgery, SCB Medical College, Cuttack, Odisha, India-753003

<sup>2</sup>PG Resident, M.K.C.G. Medical College, Berhampur, Odisha, India

<sup>3</sup>Department of Pathology, SCB Med college, Cuttack, India

## Abstract

**Aim:** Gastric cancer is the fourth common cancer and is the second leading cause of cancer-related mortality worldwide. Majority of the patients are diagnosed when the cancer is at an inoperable or metastatic stage. Median survival of patients with advanced tumors remains poor. Only 7-10 months. The best promise to improve this poor survival is provided by new agents acting against specific molecular targets. Of these, HER-2 is currently in the spotlight. Frequency of HER2 expression in gastric cancer has been reported from different geographic zones with a range of 6% to 35%. The purpose of this study was to evaluate the frequency of HER2 expression in gastric adenocarcinoma and its association with various clinicopathological characteristics and survival in Odisha.

**Methods:** A total of 150 patients undergoing gastric resection surgery (radical/palliative) for gastric adenocarcinoma at S.C.B. Medical College and hospital, Cuttack were included in this study. They were prospectively evaluated using Immunohistochemistry (IHC). HER2 overexpression was confirmed in 28 of 150 (18.7%) patients. Significantly different HER2 positivity rates were observed when comparing intestinal-type gastric cancers with diffuse/mixed-type cancers (28.6% *vs* 12.8%,  $P=0.01$ ), and moderate-differentiated cases with poorly differentiated cases (38.7% *vs* 16.7%,  $P=0.01$ ). Positive reactivity with anti cerbB-2 antibody was significantly more frequent in UICC stage-III (40%,  $p=0.005$ ). No relationship was observed between the HER2 positivity rate and sex, age, tumor site. The HER2 positive gastric cancer patients did not show statistically significant reductions in mean survival times, 1-year or 2-year survival rates. The well differentiated HER2 positive patient group exhibited shorter mean survival time (18.5 mo *vs* 27.5 mo) and lower 1-year and 2-year survival rates compared to the HER2 negative group (84.42% *vs* 96.00%; 50.65% *vs* 86.89%;  $P=0.0123$ ).

**Conclusion:** HER-2/neu overexpression is common in gastric carcinoma and more prevalent in intestinal and moderate differentiated subtypes. HER2 status has a mild impact on gastric cancer patient survival and may not constitute an independent prognostic factor in gastric cancer patients.

## Introduction

Gastric cancer is the fourth common cancer and is the second leading cause of cancer-related mortality worldwide. It causes about one million deaths worldwide per year [1]. The highest incidence of gastric cancer is seen in northeast Asia. Annual incidence rate of gastric cancer in various centers across India is 10.6 per 100,000 population. Majority of the patients are diagnosed when the cancer is at an inoperable or metastatic stage [2] and despite benefits of palliative radiotherapy & chemotherapy, survival of patients with advanced tumors remains poor (median survival 7-10 months) [3]. The best promise to improve this poor survival is provided by new agents acting against specific molecular targets. Of these, HER-2 is currently in the spotlight.

Her2/neu over-expression was first described in 1886 [4] using immunohistochemistry. 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. Her2/neu (c-erbB2) is a proto-oncogene located on chromosome 17q21 [5]. 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 [5]. HER-2 amplification and/or over expression have also been observed in colon [6], bladder [7], ovarian

[8], Fallopian tube [9], endometrium [10], lung [11], uterine cervix [12], head and neck [13], prostate [14], pancreatic [15], salivary gland [16] and esophageal [17] and gastric carcinoma [18].

A number of studies have analyzed Her2/neu overexpression in gastric carcinoma, and the rate of Her2 positivity is variable, ranging from 6% to 35%. The largest ongoing international trial which enrolled 2,992 gastric or Gastro-Esophageal Junction Cancer (GEJ), defined 21.7% of evaluable tumor samples as Her2 positive [19].

Recent evidence has shown that the HER2 plays a key role in the oncogenesis of a subset of different cancer types. Most importantly, HER2 has become a prognostic and predictive factor in breast cancer where the HER2 status is typically measured by Immunohistochemistry (IHC) or *in situ* Hybridization (ISH). Trastuzumab (Herceptin) is a monoclonal antibody which specifically targets HER2 protein by directly binding the extracellular domain of the receptor. Trastuzumab enhances survival rates in both primary and metastatic HER2-

**Correspondence to:** Dr. Sangram Keshari Panda, MS, PG Resident, Department of General Surgery, SCB Medical College, Cuttack, Odisha, India-753003, Tel: +91-9776636938; E-mail: drsangram83@gmail.com

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positive breast cancer patients has led to investigate its antitumor activity in patients with HER2 positive cancers, including gastric adenocarcinomas.

HER2 inhibition is playing a significant role as a new treatment option for gastric cancer. Numerous countries have approved the use of Herceptin for the treatment of gastric cancer and increasingly, HER2 has become a “hot” research topic.

The aim of our study was to evaluate the frequency of HER2 expression in gastric adenocarcinoma and its association with various clinicopathological characteristics and survival in Odisha.

## Methods

A consecutive prospective series of 150 patients undergoing gastric resection surgery (radical/palliative) for gastric adenocarcinoma at S.C.B. Medical College & Hospital, Cuttack, India were included in this study. Expression of HER2 in the histological specimen and correlation between the expression of HER2 & clinicopathological parameters (age, gender, tumor location, histological differentiation and pTNM classification) were evaluated in these patients.

Immunohistochemical (IHC) analysis for HER2 was performed on formalin-fixed paraffin-embedded sections of surgical specimens. Positive controls included breast cancer tissue known to exhibit high levels of her2. For scoring, Hofmann's HER2 gastric cancer scoring system was adopted. All cases showing IHC3+ were defined as HER2 positive.

The study was approved by the Institutional review board and hospital ethics committee.

## Results

The mean age was 63.6 years (from 26 to 82) and the male/female ratio was 1.46: 1 (89 men, 61 women). 12.6% of cases were stage I, 22% stage II, 23.4% stage III, and 42% stage IV. According to Lauren's classification, 56 tumors (37.3%) were intestinal, 94 (62.7%) were diffuse and mixed-type carcinomas. 23 (15%) tumors were well differentiated, 31 (20.7%) were moderate-differentiated, 96 (64%) were signet ring and poorly-differentiated. From 150 tumors, 47 were located at the body, 52 at the GEJ & Cardia and 51 were at pylorus. In 28 out of 150 samples, (18.7%) HER-2/neu protein overexpression (IHC score 3+) was observed.

## Correlation of HER2 with clinicopathological characteristics

Significantly different HER2 positivity rates were observed when comparing intestinal-type gastric cancers with diffuse/mixed-type cancers (28.6% vs 12.8%,  $P=0.01$ ), and moderate-differentiated cases with poorly differentiated cases (38.7% vs 16.7%,  $P=0.01$ ). Positive reactivity with anti cerbB-2 antibody was significantly more frequent in UICC stage-III (40%,  $p=0.005$ ). No relationship was observed between the HER2 positivity rate and sex, age, tumor site ( $P>0.05$ ; Table 1).

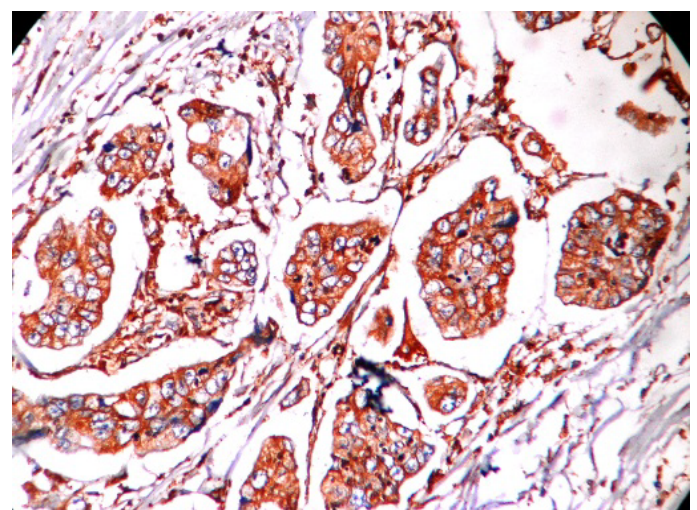
## Survival analysis

Of our 150 gastric cancer patients, 22 cases were lost in follow-up. The median survival time for the remaining 128 patients was 18 months (range: 0-33 mo). During the follow-up time, 48 deaths occurred (32%), 45 of which were disease-related. One patient died of perioperative pulmonary infection, and two cases died of heart disease and multiple organ failure, respectively. The median survival time of the HER2 positive (22 cases) and negative groups (106 cases) was 17 mo and 18.5 mo respectively. Nevertheless, the HER2 positive gastric cancer patients

**Table 1.** Correlation of human epidermal growth factor receptor-2 expression with clinicopathological characteristics.

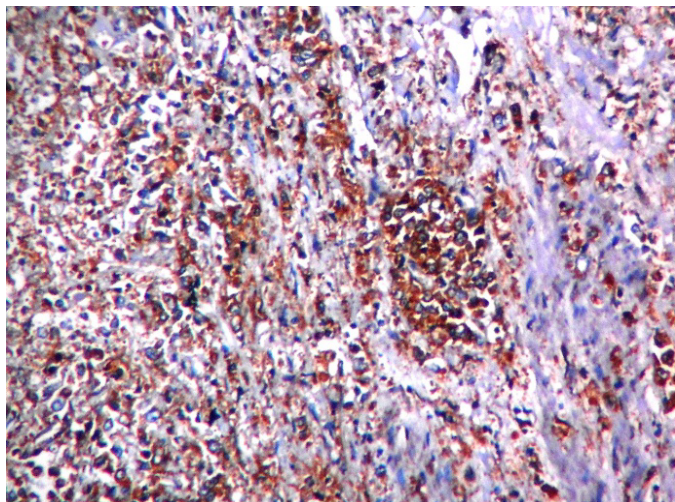
Clinicopathological characteristics	N (%)	HER-2 positive	Her-2 negative	$\chi^2$	p-value
<b>Sex</b>					
Male	89 (59%)	20 (22.5%)	69 (77.5)	1.96	0.1
Female	61 (41)	8 (13.2)	53 (86.8)		
<b>Age in yrs</b>					
<60	42 (28)	10 (23.8)	32 (76.2)	0.91	0.1
≥60	108 (72)	18 (16.6)	90 (83.4)		
<b>Tumor site</b>					
Body	47 (31.3)	10 (66.7)	37 (33.3)	0.61	0.1
GEJ & Cardia	52 (34.7)	8 (15.4)	44 (84.6)		
Pylorus	51 (34)	10 (19.6)	41 (80.4)		
<b>Lauren classification</b>					
Intestinal	56 (37.3)	16 (28.6)	40 (71.4)	5.14	0.01
Diffuse/ Mixed	94 (62.7)	12 (12.8)	82 (87.2)		
<b>Tumor Differentiation</b>					
Well diff.	23 (15.3)	0		6.21	0.01
Moderate diff.	31 (20.7)	12 (38.7)	19 (61.3)		
Signet ring & poor diff.	96 (64)	16 (16.7)	80 (83.3)		
<b>UICC Staging</b>					
I	19 (12.6)	2 (10.5)	17 (89.5)	13.85	0.005
II	33 (22)	4 (12.1)	29 (87.9)		
III	35 (23.4)	14 (40)	21 (60)		
IV	63 (42)	8 (12.6)	55 (87.4)		

\*HER2, human epidermal growth factor 2; diff., differentiated; GEJ, gastroesophageal junction.



**Figure 1.** Microphotograph showing intestinal type adenocarcinoma HER2 score 3+.

did not show statistically significant reductions in mean survival times, nor lower 1-year or 2-year survival rates. Furthermore, no statistically significant differences were observed in overall survival times between



**Figure 2.** Microphotograph showing diffuse type adenocarcinoma HER2 score 3+.

the HER2 positive and negative groups ( $\chi^2=0.9157$ ,  $P=0.3386$ ). Within the well differentiated gastric cancer patient group, patients with HER2 tumor positivity had poorer outcomes than those with HER2 negative tumors. The well differentiated HER2 positive patient group exhibited shorter mean survival time (18.5 mo vs 27.5 mo) and lower 1-year and 2-year survival rates compared to the HER2 negative group (84.42% vs 96.00%; 50.65% vs 86.89%;  $P=0.0123$ ). The median survival time of the HER2 positive group did not show any statistical associations when compared to the subgroups of sex, age, tumor site, TNM classification, depth of invasion, lymph node metastases and distant metastasis in gastric cancer. Within the poorly differentiated and diffuse/mixed type gastric cancer patient groups, no statistically significant differences were observed between the HER2 positive and HER2 negative groups.

## Discussion

In the present study, IHC scoring criteria followed that of Hofmann [20] which is considered to be the most appropriate HER2 scoring system in human gastric cancer. Furthermore, to ensure the reliability of our results, we followed the guidelines on HER2 detection in breast cancer, recommended by the American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP) [21].

Herceptin (trastuzumab) is a recombinant human monoclonal antibody designed to target and block the function of HER2 by directly binding to the extracellular domain of the receptor [22,23]. It has been used for the treatment of HER2 overexpressing breast cancer for more than 10 years and was approved by the European Medicines Agency [24] in 2010 for use in combination with capecitabine or 5-FU and cisplatin for metastatic gastric or GE junction cancers, based on data from the "ToGA" clinical trial. The exact anti-tumor mechanism of Herceptin is not fully understood, however some mechanisms have been postulated [23,25-29] including interruption of HER2 mediated cell signaling pathways and cell cycle progression; induction of antibody-dependent cell-mediated cytotoxicity and apoptosis; induction of anti-angiogenesis effects and increasing receptor turnover by endocytosis. As clinical surgeons, we should be readily and accurately able to identify which patients are suitable for Herceptin treatment. An accurate and reliable HER2 scoring system, together with clinical information, may help us to better determine whether a gastric cancer patient is a potential candidate for targeted therapy using Herceptin.

The relationship between *HER2* gene amplification and protein expression in gastric cancer patients is controversial [30,31]. Nevertheless, more recent studies have reported a high concordance between gene amplification and protein overexpression using FISH and IHC approaches [32-34]. Indeed, the ToGA trial [35] (which recruited the largest population of gastric cancer patients to date-3807) reported a HER2 FISH and IHC concordance rate of 87.5%, and further reported that HER2 IHC3+ cases were almost all entirely *HER2* gene amplified (97.5% of cases) [29]. In our study, HER2/neu status was determined by immunohistochemistry and all IHC 3+ tumors are accepted as HER2/neu positive cases. Out of 150 cases 28 cases (18.7%) show HER2/neu 3+, 122 cases were HER2/neu negative or HER2/neu 1+ and no cases showed HER2/neu 2+ score. In our study, no relationship was observed between HER2 positivity and sex, age and tumor site ( $P>0.05$ ). However, intestinal-type and moderate-differentiated gastric cancer cases showed a higher HER2 positive rate than diffuse/mixed-type and poorly-differentiated cancer cases respectively. Of interest, the ToGA trial reported a higher HER2 positivity rate in GE junction cancers compared to other gastric cancers (33.2% vs 20.9%,  $P<0.001$ ) [36]. Our study, as well as that of another group [37], showed no statistically significant difference between HER2 positivity and the gastric tumor site.

A review of 35 published studies, which evaluated the prognostic value of HER2, indicated no differences in the majority of studies, with regard to Overall Survival (OS). Two studies identified a longer OS, while 13 (37%) observed a significantly poorer OS [38]. Whilst our study did not show any correlation between HER2 status and overall survival, patients with well-differentiated HER2 positive tumors showed poorer survival times compared to patients with HER2 negative tumors. We speculate that HER2 status has a mild impact on gastric cancer patient survival and may not constitute an independent prognostic factor in gastric cancer patients. Clearly, further research is required to explain the impact of HER2 on development and prognosis of gastric cancer.

## Conclusion

HER2 inhibition is playing a significant role as a new treatment option for gastric cancer. Numerous countries have approved the use of Herceptin for the treatment of gastric cancer. To date, there have been limited studies to determine any correlations of HER2 expression with clinicopathological characteristics and prognosis in Indian patients with resectable gastric cancer. Intestinal type, moderate-differentiated and Stage-III gastric cancer patients showed a higher HER2 positivity rate and thus could represent ideal candidates for targeted-therapy using Herceptin. HER2 status has a mild impact on gastric cancer patient survival and may not constitute an independent prognostic factor.

## Limitation of our study

The gold standard test (FISH) could not be done, as was not available in our setup, for confirmation of HER2/neu overexpression.

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