### **Original Research Article**

DOI: http://dx.doi.org/10.18203/2349-2902.isj20171627

# Incidence of human epidermal growth factor receptor-2 expression and its correlation with clinicopathological characteristics in gastric cancer at tertiary care centre at IGMC Shimla, Himachal Pradesh, India

Vishal Thakur<sup>1</sup>, Puneet Mahajan<sup>1</sup>, Neelam Sharma<sup>2</sup>, Ved Kumar Sharma<sup>1</sup>, Tushar Patial<sup>1</sup>, Biplav Singh<sup>1\*</sup>, Nidhi Raina<sup>2</sup>

**Received:** 09 March 2017 **Accepted:** 04 April 2017

## \*Correspondence: Dr. Biplay Singh,

E-mail: drbiplavsingh@gmail.com

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

#### **ABSTRACT**

**Background:** Trastuzumab has been approved for the treatment of patients with HER-2 positive metastatic/locally advanced adenocarcinoma of the stomach by FDA. Most of gastric cancer patients present at an advanced stage in our setup, all patients of gastric adenocarcinoma should be subjected to HER-2 testing at initial diagnosis. Immunohistochemistry should be the initial testing methodology and Fluorescence in-situ hybridization should be used to retest immunohistochemistry 2+ samples.

**Methods:** We conducted this study with the aim to establish the expression profile of HER-2 and its correlation to clinicopathological characteristics in resectable cases of gastric adenocarcinoma, in patients presenting with gastric adenocarcinoma at IGMC Shimla. The study was conducted over a year from 1st July 2014 to 30th June 2015. Sample size included 40 diagnosed cases of resectable gastric adenocarcinoma. All patients with operable and resectable disease were subjected to surgery and the specimen were sent for detailed histopathological examination and immunohistochemistry.  $\chi^2$  statistical analysis was performed to assess the HER-2 positivity rate. All statistics were performed using 2-sided analysis, with a significance level of p< 0.05.

**Results:** In present study, 7 cases (17.5%) were scored as strongly positive for HER-2 membrane staining (3+), 4 cases (10%) were moderately positive (2+) and 29 cases (72.5%) were HER-2 negative (0/1+) on IHC. The positivity rates in diffuse type (22.2%) and poorly-differentiated (25%) gastric cancer were higher than those in intestinal type (9.9%) and well-differentiated (14.3%) gastric cancer respectively.

**Conclusions:** There is a strong association of human epidermal growth factor receptor-2 positivity with Gastric Malignancy in our country especially in poorly differentiated cancers. Targeted biological therapy with Trastuzumab hence becomes an attractive addition to the multimodality therapy usually required in the treatment of locally advanced /metastatic gastric cancers.

Keywords: Epidermal growth factor receptor-2, EGFR 2, HER 2, Trastuzumab, Gastric cancer

#### INTRODUCTION

Gastric cancer is the 2nd most common cause of cancerrelated deaths in Indian population. In India, the number of new gastric cancer cases, annually, is approximately 34,000 with a male predominance (male-to-female ratio, 2:1) with a progressive increase postulated such that by the year 2020, there would be approximately 50,000 new gastric cancer cases annually in India.<sup>1,2</sup> The prognosis for most patients with gastric cancer is poor, with 5-year

<sup>&</sup>lt;sup>1</sup>Department of Surgery, Indira Gandhi Medical College and Hospital, Shimla, Himachal Pradesh, India <sup>2</sup>Department of Pathology, Indira Gandhi Medical College and Hospital, Shimla, Himachal Pradesh, India

survival for all patients less than 30%.3 Given the disappointing results of standard therapy, a paradigm shift in oncology is occurring that focuses on tailoring therapy to the individual patient. Trastuzumab based therapy has been shown to confer overall survival benefit in HER-2 positive patients with advanced gastric cancer in large multi centric trial (ToGA trial).4 In this multiinstitutional international study, 22.1% of screened patients with advanced or metastatic gastric cancer were HER-2 positive and randomized to either therapy with a HER-2-targeted monoclonal antibody (trastuzumab) in addition to standard chemotherapy or standard chemotherapy alone. The trial demonstrated a significant increase in median overall survival of 2.7 months for patients receiving trastuzumab (13.8 versus 11.1 months. HR 0.74, 95% CI [0.60-0.91], p=0.0046) and led to the FDA approval of the drug as part of first line therapy for patients with metastatic gastric cancer. Finally, in the ToGA trial, HER-2 positivity differed significantly by histological subtype (intestinal 34%, diffuse 6%, mixed 20%).

ToGA trial was noteworthy both in its successful introduction of a new therapeutic agent for patients with advanced/metastatic gastric cancer and in its contribution to the standardization of HER-2 testing (based on study by Hofmann et al) in all stages of gastric cancer.

Given the paucity of studies on HER-2 overexpression in gastric cancer in our country, we conducted this study with the aim to establish the expression profile of HER-2 and its correlation to clinicopathological characteristics in resectable cases of gastric adenocarcinoma, in patients presenting with gastric adenocarcinoma at IGMC Shimla.

#### **METHODS**

The study was conducted after ethics committee approval (Himachal Pradesh University, Shimla, India) from 1st July 2014 to 30th June 2015. Sample size included 40 diagnosed cases of resectable gastric adenocarcinoma. Written and informed consent was taken from every patient.

All patients with operable and resectable disease were subjected to surgery and the specimen were sent for detailed histopathological examination and immunohistochemistry in 10% neutral buffered formalin to department of pathology. Their 10% neutral buffered formalin fixed paraffin embedded (FFPE) tissue blocks were retrieved. Sections were made and staining done using routine haematoxylin and Eosin stain. The slides were reviewed for quality and quantity of material by a pathologist IGMC, Shimla laboratories. Suitable cases were selected and presented for IHC for HER-2 receptors using anti HER-2 antibodies.

All histopathological diagnosis was established on routine haematoxylin and eosin staining of the sections. Immunohistochemistry for HER-2 was done on Bio

Genex Xmatrx fully automated front-end processing system.

Immunohistochemistry staining 5 - IHC staining was done on fully automated Xmatrx IHC staining machine on poly-l-lysine coated slides from formalin fixed, paraffin embedded tissues. 3-4 microns' thin sections were mounted on slides. Slides were then placed in fully automated Xmatrx IHC staining machine, where the slides were heated at 800°C for 15-20 minutes. Dewaxing was done for 3 cycles of 3 minutes each and washed with IHC buffer solution.

Antigen retrieval was done by applying micro reagent: EZAR 1 (HX0031) for HER-2, the slides were heated for 800°C for 20-25 minutes and washed with IHC buffer. Peroxide block was then applied (HX0026) and slides were incubated for about 10 minutes. Power block was then applied (HX0083) and slides incubated for about 10 minutes. Antibody was added for HER-2. Slides were heated and incubated at 370°C for 1 hour, washed with IHC buffer and deionized water. Slides were heated and incubated at 250°C for 40 minutes, washed with IHC buffer and deionized water. Polymer HRP was added to slides and were heated at 300C and incubated for 30 minutes. DAB working solution was added and slides incubated for 8-10 minutes and then washed with deionized water and IHC buffer. Counterstaining was then done with haematoxylin and slides incubated for 5 minutes and then washed with deionized water and IHC buffer. Mounting was done by using DPX mountant. HER-2 overexpression was correlated clinicopathological profile of the tumours.  $\chi$  2 statistical analysis was performed to assess the HER-2 positivity amongst the subgroups with different clinicopathological characteristics. All statistics were performed using 2-sided analysis, with a significance level of p<0.05.

#### **RESULTS**

In this prospective study, the mean age of patients with carcinoma was 60 years (10.9 years SD). 55% of patients were greater than 60 years of age and 45 % of patients were less than 60 years of age (p = 0.9735). The number of male patients were more than female (67.5% vs 32.5%, p = 0.4604). According to Hofmann's HER-2 gastric cancer scoring criteria, 7 cases (17.5%) were scored as strongly positive for HER-2 membrane staining (3+), 4 cases (10%) were moderately positive (2+) and 29 cases (72.5%) were HER-2 negative (0/1+) on IHC. Most of the tumors were located at antrum (22; 55%) and the number of patients involving cardia and body were 2 (5%) and 15 (37.5%) respectively. One patient had diffuse involvement of entire stomach like linitisplastica. The HER-2 positivity rate was not correlated with tumor location (p=0.1314). The histopathological examination revealed Lauren's diffuse variant as most common pathology in 27 (67.5%) patients. Most of them had poorly differentiated adenocarcinoma (24. 60%). The positivity rates in diffuse type (22.2%) and poorly-differentiated (25%) gastric cancer was higher than those in intestinal type (9.9%) and well-differentiated (14.3%) gastric cancer respectively. However, HER-2 overexpression rate was not correlated with histological

types or tumour differentiation (p >0.05). Among all patients, 33 were staged as T4, 3 were staged T3, 2 each were staged T2 and T1. HER2 overexpression was also not correlated with T-stage (p = 0.1544).

Table 1: correlation of HER2 with clinicopathological characteristics.

Clinicopathologic characteristics	n	HER-2			2	n volue
		Positive	Equivocal	Negative	χ2	p value
Age (years)						
<60	18	03 (3.15)	02 (1.80)	13(13.05)	0.0537	0.9735
>60	22	04 (3.85)	02 (2.20)	16 (15.95)		0.9733
Gender						
Male	27	06 (4.72)	03 (2.70)	18 (19.58)	1.5511	0.4604
Female	13	01 (2.28)	01 (1.30)	11 (9.43)		0.4604
Tumour site						
Cardia	02	00 (0.86)	00 (0.38)	02(2.72)	9.8424	
Body	15	03 (3.21)	00 (1.43)	12 (10.36)		0.1214
Antrum	22	03 (4.71)	04 (2.10)	15 (15.19)		0.1314
Linitis plastica	01	01 (0.21)	00 (0.10)	00 (0.69)		
Lauren's classification						
Intestinal	11	01 (1.92)	01 (1.10)	09 (7.98)	1.848	
Diffuse	27	06 (4.72)	03 (2.70)	18 (19.58)		0.7637
Mixed	02	00 (0.35)	00 (0.20)	02 (1.45)		
Tumour differentiation						
Well-differentiated	07	01 (1.23)	01 (0.13)	06 (5.08)	4.837	
Moderately-differentiated	09	00 (1.58)	00 (0.90)	09 (6.52)		0.3044
Poorly-differentiated	24	06 (4.20)	03 (0.15)	18 (17.43)		
T-stage						
T1	02	00 (0.35)	00 (0.20)	02 (4.95)	9.3574	
T2	02	01 (1.21)	01 (0.20)	00 (28.05)		0.1544
Т3	03	00 (0.52)	01 (0.30)	02 (2.17)		0.1544
T4	33	06 (5.78)	02 (3.30)	25 (23.92)		

#### Gastric adenocarcinoma - IHC staining

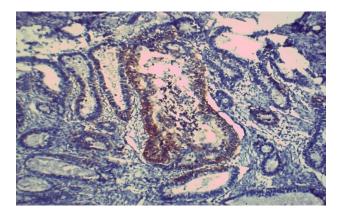


Figure 1: Well-differentiated adenocarcinoma, HER-2 score 3+ (IHC, 100X)

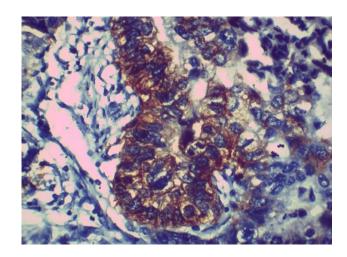


Figure 2: Well-differentiated adenocarcinoma, HER-2 score 3+ (IHC, 400X)

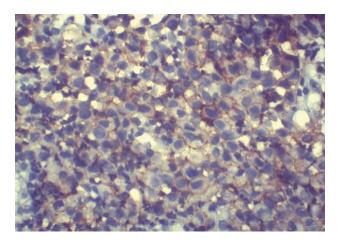


Figure 3: Poorly -differentiated adenocarcinoma, HER-2 score 3+ (IHC, 400X).

Table 2: HER-2 expression.

Variable	Frequency (%)
IHC 3+ (positive)	07 (17.5)
IHC 2+ (equivocal)	04 (10)
IHC 1+ (negative)	03 (7.5)
IHC 0 (negative)	26 (65)
Total	40

#### **DISCUSSION**

The HER-2 gene is a proto-oncogene located on chromosome 17.6. It expresses HER-2 protein, one of the epithelial growth factor receptor families, and has tyrosine kinase activity, which mediates cancer proliferation. HER-2 gene amplification and protein overexpression in gastric cancer were first reported in 1986 and have since been confirmed by numerous studies, highlighting ranges in both HER-2 gene amplification rates from 16%-27.1% by FISH analysis and HER-2 protein overexpression from 4%-53% by IHC analysis. The variability within these results is likely due to several factors including sample size, study design and differences in geographic location. However, the most important variability factor is likely a consequence of having no standardized HER-2 test and scoring criteria.8 In the present study, IHC scoring criteria followed were that of Hofmann which is considered to be the most appropriate HER-2 scoring system in human gastric cancer.<sup>9</sup> Furthermore, to ensure the reliability of our results, we used the test kit certified by the United States Food and Drug Administration. Herceptin (trastuzumab) is a recombinant human monoclonal antibody designed to target and block the function of HER-2 by directly binding to the extracellular domain of the receptor.<sup>10</sup> Herceptin has been used for the treatment of HER-2 overexpressing breast cancer for more than 10 years and was approved by the European Medicines Agency 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.1

The exact anti-tumor mechanism of Herceptin is not fully understood. However, some mechanisms have been postulated including interruption of HER-2 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.<sup>11</sup>

The incidence of HER-2 overexpression in our study was 17.5% and this is in comparable with other studies. (ToGA trial 2 - 22.1%, Chao et al - 25.38%, Shan et al - 9.8%, Yan et al - 11.7%, Yonemura et al - 11.9%, Rajagopal I et al - 26.7% Patil P et al - 7%, Lakshmi V et al - 35.9%).<sup>2,8,13-17</sup>

The mean age of patients with carcinoma in our study was 60 years (10.9 years SD HER-2 was overexpressed in 18.2% (3/18) patients under 60 years of age and in 16.7% (4/22) patients older than 60 years. This is not statistically significant with no association between HER-2 overexpression and age which is also seen in other studies.<sup>2,8,12-17</sup>

Amongst our study subjects, gastric cancer was more common in males (67%, n = 27/40) than in females (33%, n = 13/40). This correlates with a distribution ratio of 2:1 as seen in the literature. However, HER-2 overexpression was 22.2% (n=6/27) in males and 7.7% (n = 1/13) in females, showing no significant association of HER-2 overexpression and gender. Similar trend is observed in some studies. Begographic and ethnic heterogeneity of tumour associated aberration which exist in solid tumours may help to explain the differences for HER-2 overexpression in various studies. In addition, there is paucity of data in our Indian population for HER-2 overexpression with no specific documented prevalence in Indian population.

Specimens which demonstrated weak to moderate complete or basolateral membranous reactivity in more than 10% of cells (10%, N = 4/40) were classified as equivocal (+2). These cases required to undergo FISH test evaluation to classify them as positive or negative for HER-2 overexpression. FISH was not available in our study as alluded earlier in the study limitation. However, in the ToGA trial 26% of the equivocal were FISH positive for HER-2 overexpression, while in a Chinese cohort study, 28.8% of equivocal turned positive upon FISH evaluation. On extrapolation using the above studies, our HER-2 positivity will be expected to rise to 22.5%.

On assessment of HER-2 overexpression in specific anatomical sites, only tumours located at body and antrum overexpressed HER-2. Further, it was observed that 20% (n = 3/15) of tumours located at body and 13.6% (n = 3/22) of gastric cancer located at antrum, overexpressed HER-2. Present study, as well as that of another group, showed no statistically significant difference between HER-2 positivity and the gastric

tumor site. In general, out of all the 7 cases which revealed HER-2 over-expression, 3 cases each were located at body and antrum (15%) and in remaining one case (2.5%) there was diffuse involvement of stomach. However, tumours located at cardia (5%, 2/40) did not overexpress HER-2 and this association was not statistically significant.<sup>2,8,12-17</sup> This was attributed to fewer cases of cardia cancer in our study.

On evaluation of histological pattern for HER-2 overexpression, this study shows higher HER-2 overexpression in diffuse type (22.2%, n =6/27) compared to intestinal (9.9%, n = 1/11) and mixed (0%, n = 9/40) for gastric adenocarcinoma. This is in contrast to other studies comparing HER-2 overexpression and histological types of gastric adenocarcinoma.  $^{2,8,12-17}$  This could be attributed to higher prevalence of diffuse type of gastric cancer in our sample population. However, this association was not statistically significant and this trend is similar to trend in other studies.

In present study, HER-2 overexpression was seen more in poorly-differentiated cancers (25%, n = 6/24 as compared to well-differentiated cancers (14.3%, n = 1/7). However, this association was not statistically significant. This finding is in contrast to other studies which demonstrated both an association and no association between HER-2 overexpression and tumor differentiation. <sup>2,8,12-17</sup> These conflicting data may be due to different sample sizes and the low prevalence of HER-2 in GC. In addition, varying methods of evaluation and scoring schemes with different cut-points were used before the establishment of a standard guideline for assessing HER-2 expression. Perhaps with the current consistent guidelines for HER-2 assessment in this disease, future studies will provide more clarity regarding this issue.

Most of cases in our study were pathologically T4 and HER-2 expression was 18.2% (n = 6/33) in T4 tumours. Out of the two cases with T2 tumours, HER-2 overexpression was seen in half. However, this association was also not statistically significant which is concordance with other studies.  $^{2,8,12-17}$ 

#### **CONCLUSION**

As clinical surgeons, study should be readily and accurately able to identify which patients are suitable for Herceptin treatment. In view of the high cost of treatment with trastuzumab therapy and different HER-2 staining pattern in gastric cancer from that in breast cancer due to tumour heterogenicity, HER-2 testing should be standarised and interpretation of HER-2 results is performed with strict adherence to the scoring criteria specific for gastric cancer to select eligible candidates for trastuzumab therapy. Immunohistochemistry should be the initial testing methodology and Fluorescence in-situ hybridization should be used to retest immunohistochemistry 2+ samples. Hence, the need to build our local capacity for Fluorescence in-situ

hybridization test. Further studies with larger cohorts need to be conducted to provide more clarity on prevalence of HER-2 overexpression and to explore the role of HER2 as an independent prognostic factor in our set up.

Funding: No funding sources Conflict of interest: None declared

Ethical approval: The study was approved by the

institutional ethics committee

#### **REFERENCES**

- 1. Trastuzumab LF. A new treatment option for HER2positive metastatic gastric and gastroesophageal junction cancer. Future Oncol. 2011;7:187-99.
- Shrikhande SV, Sirohi B, Barreto SG, Chacko RT, Parikh PM, Pautu J, et al. Indian Council of Medical Research consensus document for the management of gastric cancer. Indian J Med Paediatr Oncol. 2014;35:239-43.
- 3. Patel SH, Kooby DA. Gastric adenocarcinoma surgery and adjuvant therapy. Surg Clin North Am. 2011;91(5):1039-77.
- 4. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. The Lancet. 2010;376(9742):687-97.
- 5. Choudary KG, Yagle KJ, Swanson PE, Krohn KA, Rajendran JG. A robust automated measure of average antibody saining in immunohistochemistry images. J Histochem Cytochem. 2010;58:95-107.
- 6. Rubin I, Yarden Y. The basic biology of HER2. Ann Oncol. 2001;12(Suppl1):S3-8.
- 7. Sakai K, Mori S, Kawamoto T, Taniguchi S, Kobori O, Morioka Y, et al. Expression of epidermal growth factor receptors on normal human gastric epithelia and gastric carcinomas. J Nat Cancer Institute. 1986;77(5):1047-52.
- 8. He C, Bian XY, Ni XZ, Shen DP, Shen YY, Liu H, et al. Correlation of human epidermal growth factor receptor 2 expression with clinicopathological characteristics and prognosis in gastric cancer. World J Gastroenterol. 2013;19(14):2171-8.
- 9. Hofmann M, Stoss O, Shi D, Büttner R, van de Vijver M, KimW, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathol. 2008;52:797-805.
- 10. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release. 2010;146:264-75.
- Hudis CA. Trastuzumab -mechanism of action and use in clinical practice. N Engl J Med. 2007;357:39-51
- 12. Shan L, Ying J, Lu N. HER2 expression and relevant clinicopathological features in gastric and

- gastroesophageal junction adenocarcinoma in a Chinese population. Diagnost pathol. 2013;8(1):76.
- 13. Yan B, Yau EX, Bte Omar SS. A study of HER2 gene amplification and protein expression in gastric cancer. J Clin Pathol. 2010;63:839-42.
- 14. Yonemura Y, Ninomiya I, Yamaguchi A, Fushida S, Kimura H, Ohoyama S, et al. Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res. 1991;51(3):1034-8.
- 15. Rajagopal I, Niveditha SR, Sahadev R, Nagappa PK, Rajendra SG. HER 2 expression in gastric and gastro-esophageal junction (GEJ) adenocarcinomas. Journal of clinical and diagnostic research: JCDR. 2015;9(3):EC06-10.
- 16. Patil PS, Mehta SA, Mohandas KM. Overexpression of HER2 in Indian patients with gastric cancer. Indian J Gastroenterol. 2012:1.
- 17. Lakshmi V, Valluru VR. Role of her 2 neu in gastric carcinoma-3 year study in a medical college hospital. Indian J Appl Res. 2014;4(11).
- 18. Yan SY, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X, et al. Clinicopathologic significance of HER-2/neu

- protein expression and gene amplification in gastric carcinoma. World J Gastroenterol. 2011;17(11):1501-6.
- 19. Johansson B, Mentens P, Miteiman P. Geographic heterogeneity of neoplasia associated chromosome aberration. Genes chromosome Cancer. 1991:3:1-7.
- Shan L, Ying J, Lu N. HER2 expression and relevant clinicopathological features in gastric and gastroesophageal junction adenocarcinoma in a Chinese population. Diagnost pathol. 2013;8(1):76.
- 21. Christa L. Whitney-Miller, David G. HER2 Testing in gastric and oesophageal adenocarcinoma: emerging therapeutic options and diagnostic challenges. Connection. 2010;15:49-50.

Cite this article as: Thakur V, Mahajan P, Sharma N, Sharma VK, Patial T, Singh B, et al. Incidence of human epidermal growth factor receptor-2 expression and its correlation with clinicopathological characteristics in gastric cancer at tertiary care centre at IGMC Shimla, Himachal Pradesh, India. Int Surg J 2017;4:1715-20.