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Significance of Her 2 Gene Expression in Gastric Cancer Patients- A Retrospective Study

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ABSTRACT

Amplification of *HER2* gene is an important biomarker for eligibility for *HER2* targeted therapies. In the present study, the frequency of *HER2* expression and its relationship with clinicopathological features were examined in gastric cancer patients.

Histopathologically proven newly diagnosed 31 gastric cancer patients were included for Fluorescence in Situ Hybridization (FISH) study. FISH was performed on paraffin embedded tissues using ZytoLight SPEC *HER2* /CEN 17 Dual Color Probe Kit. ASCO/CAP guidelines were followed for analysis. The correlation between FISH results and clinicopathologic variabes were studied.

Total 31 patients were enrolled in the study, among that 19 patients (61%) were male and 12 patients (39%) were females. The median age was 52 years with 30 to 72 years age range. *HER2* gene amplification was found in 45% (14/31) of the patients. Higher rate of *HER2* positivity was significantly associated with moderately and poorly differentiated adenocarcinoma ($p \leq 0.05$).

FISH allows rapid visual and accurate assessment of the *HER2* gene amplification on histological sections. Strength of the present study is higher rate of *HER2* positivity in moderately and poorly differentiated types of adenocarcinoma which explains that the bad prognosis is associated with *HER2* positivity. Detection of *HER2* amplification will help to identify the specific subsets of patients who might benefit from molecular targeted therapies like trastuzumab.

KEY WORDS: FISH, *HER2*, Gastric cancer

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INTRODUCTION

Human Epidermal Growth Factor receptor 2 (*HER2*) located on long arm of chromosome 17, encoding 185kd *HER2* gene is related to the oncogene v-erbB of the avian erythroblastosis virus and located adjacent to the Topoisomerase IIa genes[1]. It is transmembrane receptor, tyrosine kinase and the member of epidermal growth factor receptor (EGFR) family, having an important role in cell growth, differentiation and survival[2].

HER2 function as an oncogene in carcinomas, because amplification of *HER2* leads to protein over expression on the cell membrane and subsequent acquisition of advantageous properties for malignant cells [3]. It has been found to be over expressed in various type of malignancies, notably breast[4], ovarian[5], lung cancer[6], prostate[7], colorectal[8] and gastric cancer[9]. *HER2* is well identified as a key factor in the development of breast cancer in which it confers poor prognosis and predicts for response to *HER2* targeted therapy. In addition, *HER2* testing is also applied in gastric cancer. As stated in the literature *HER2* positivity rates vary from 4–53% in gastric cancer[2,9,10]. Globally gastric cancer is the fifth most common neoplasm and third leading cause of death[11]. Targeted therapies are a newer trend in cancer treatment to improve overall survival in patients with cancer. Trastuzumab, is a recombinant humanized monoclonal antibody targeting *HER2* gene with two antigen specific sites that bind to the juxtamembrane portion of the extracellular domain of the *HER2* receptor, thereby preventing activation of its intracellular tyrosine kinase. In in- vitro and in-vivo models of *HER2* positive gastric cancer, trastuzumab has shown significant antitumor effect[12]. Moreover, results from the international, randomized, Phase III Trastuzumab for Gastric Cancer (ToGA) study has demonstrated a survival benefit with trastuzumab plus chemotherapy (capecitabine or 5-fluorouracil and cisplatin) in patients with *HER2* -positive locally advanced, recurrent and/or metastatic gastric tumors that overexpress *HER2*. Patients with high *HER2* expressing tumors get the greatest benefit from trastuzumab therapy [13]. Therefore, precise estimation of *HER2* levels in gastric cancer is required before application of specific therapeutic agent. Considering these facts, the aim of the present study was to evaluate *HER2* gene amplification by Fluorescence in Situ Hybridization (FISH) in gastric cancer and correlation with clinicopathological parameters.

MATERIALS AND METHODS

In the present retrospective study, 31 paraffin-embedded tissue samples of biopsy specimens of gastric adenocarcinoma cases were selected. The patients' medical records were reviewed to obtain patients' clinicopathological details, including age at diagnosis, gender, tumor differentiation, presence of signet cells, tumor location and lymph node involvement.

FISH analysis was carried out using commercially available ZytoLight SPEC *ERBB2* /CEN 17 Dual Color Probe Kit (ZytoVision GmbH, Germany). It contains two fluorochrome-labelled DNA probes, ERBB2 (*HER2*) labelled with Spectrum Green and CEN17 probe labelled with Spectrum Orange. FISH assay was performed as per the manufacturer's instructions. In brief, tumor tissue sections were deparaffinized, dehydrated with alcohol series (100%, 90%, 70%), incubated in pre-warmed heat pre-treatment solution (PT1) at 90º C. Drop wise Pepsin Solution (ES1) was applied to the tissue section and incubated for 16 min at 37 ºC in a humidity chamber. Washes were carried out in Wash Buffer (WB1) for 5 min and 1min in TDW at RT. Subsequently appropriate amount of probe was applied on tissue section followed by denaturation and hybridization process which was carried out overnight at 37°C in a humid chamber, Post-hybridization washes were carried out in 1X Wash Buffer and dehydrated with alcohol series, slides were counterstained with DAPI. The slides scanning and capturing were done using OLYMPUS BX 51 fluorescent microscope (OLYMPUS BX51, Japan). Images were captured at a magnification of 100X, Total 20 randomly selected invasive tumor cells were evaluated for interpretation. In case of equivocal result, more tumor nuclei were evaluated by blinded evaluation. The ratio of *HER2* signals to centromere CEN17 signals were calculated and ASCO/CAP guidelines were used for analysis and interpretation of results[14]. Statistical analysis of the data was performed using SPSS 20.0 statistical software. The chi square test was used to assess the relationship between *HER2* status and clinicopathological features. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

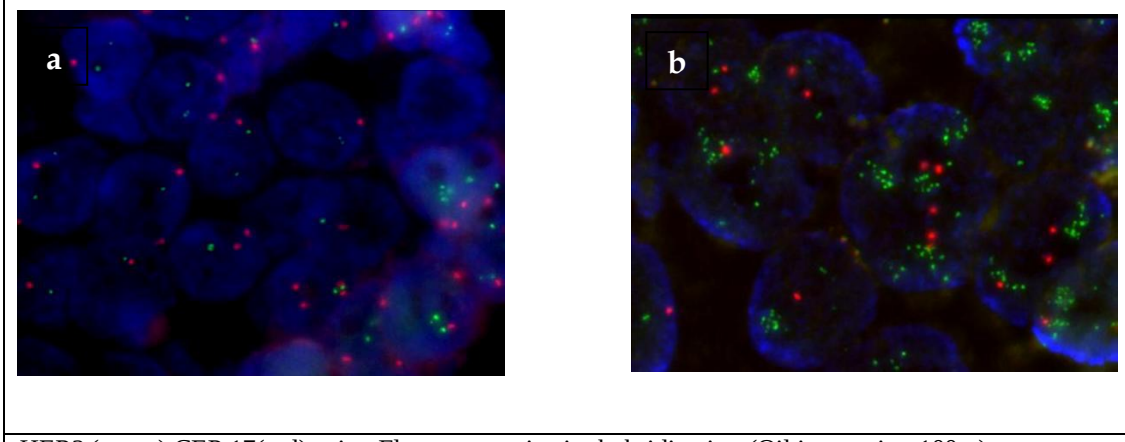
HER2 gene expression was examined in 31 paraffin-embedded tissue samples of gastric adenocarcinoma patients. Eligibility criteria included patients with newly diagnosed and histologically confirmed advanced or/metastatic gastric cancer. Tumor samples were obtained prior to any chemotherapy or systemic treatment.

Table1 shows clinicopathological characteristics of gastric cancer patients. All patients had adenocarcinoma; mean age of patients was 52 years. Among 31 patients, 16 (52%) had poorly differentiated tumors, 8 (26%) had moderately differentiated tumors, 1 (3%) had well differentiated and 6 (19%) were observed with unknown for tumor differentiation status. 21 (68%) patients had tumor at distal stomach site, 6 (19%) patients had tumor at proximal stomach site, and 4 (13%) patients with GEJ/esophagous tumor location. The lymph nodes were present in 12(39%) and absent in 19 (61%) patients.

Table No. 1: Clinicalpathological Characteristics of total 31 Gastric cancer patients

Variables	**Number of Patients (%)**
Age range	30-72
≤ 45	11(35%)
>45	20 (65%)
Gender	
Male	19 (61%)
Female	12 (39%)
Tumor Differentiation	
Well	1 (3%)
Moderately	8 (26%)
Poorly	16 (52%)
Unknown	6 (19%)
Signet ring cells	
Present	8 (26%)
Absent	23 (74%)
Tumor Location	
GEJ/esophagous	4 (13%)
Proximal stomach	6 (19%)
Distal stomach	21 (68%)
Lymphnode status	
Presence	12 (39%)
Absent	19 (61%)
Treatment	
Chemotherapy	12 (39%)
Chemotherapy+Surgery	4 (12%)
Chemotherapy+Radiotherapy	4 (12%)
Surgery	7 (22%)
None	4 (12%)

Out of the 31 patients, 14 (45%) patients were scored as *HER2* positive. Using the composite definition of *HER2* positivity (i.e., Positive ratio ≥ 2 or ratio <2 and average *HER2* copy numbers ≥ 6 signals/cell and Negative ratio <2 and average *HER2* copy number<4 signals/cell) [**Fig1.**]



HER2 (green) CEP 17(red) using Fluorescence in situ hybridization (Oil immersion 100x.)

a: *HER2*/CEP17- Negative; Tumor cells showing no amplification of *HER2*

b: *HER2*/CEP17- Positive; Tumor cells with highly amplified *HER2*

Figure 1: Representative image of FISH results for *HER2* gene on paraffin embedded tissue

TABLE No. 2: Correlation between *HER2* gene status and clinicopathological characteristics

Variables	***HER2* by FISH Positive (n=14)**	***HER2* by FISH Negative (n=17)**	**p value**
Age ≤ 45 (n=11)	5 (45%)	6 (55%)	
Age >45 (n=20)	9 (45%)	11 (55%)	0.66
Gender			
Female (n=12)	4 (33%)	8 (67%)	0.29
Male (n=19)	10 (53%)	9 (47%)	
Tumor differentiation			
Well (n=1)	0	1	0.05
Moderately (n=8)	4 (50%)	4 (50%)	
Poorly (n=16)	10 (63%)	6 (37%)	
Unknown (n=6)	0	6 (100%)	
Signet ring cells			
Present (n=8)	4 (50%)	4 (50%)	0.11
Absent (n=23)	10 (43%)	13 (57%)	
Tumor location			
GEJ*/esophagous (n=4)	2 (50%)	2 (50%)	0.97
Proximal stomach (n=6)	3 (50%)	3 (50%)	
Distal stomach (n=21)	9 (43%)	12 (57%)	
Lymphnode Involvement			
Yes (n=12)	7 (58%)	5 (42%)	0.40
No (n=19)	7 (37%)	12 (63%)	
GEJ*: Gastroesophageal junction			

Clinicopathological characteristics and their association with *HER2* gene status are shown in table 2. Out of total 31 gastric cancer patients, 20 patients were more than 45 years age group, out of which 9 (45%) patients were *HER2* positive. While, 11 patients were less than 45 years age group, out of which 5 (45%) patients were *HER2* positive. *HER2* was positive in 4 (33%) out of 12 female patients and in 10 (53%) out of 19 male patients. Notable difference was not obtained between *HER2* gene amplification with age and gender. In present study, *HER2* gene amplification also correlated with tumor differentiation. Out of 16 poorly differentiated cases, 10 cases (63%) were positive for *HER2* gene amplification. While 4 cases (50%) out of 8 moderately differentiated tumors type showed *HER2* gene amplification, none of the patients had *HER2* positivity in well and unknown tumor differentiation type i.e. negative for *HER2* gene amplification. The difference was statistically significant ($p \leq 0.05$). Likewise, frequency of *HER2* amplification was higher in distal stomach location (43%) as compared to tumor found at GEJ and proximal stomach site. Out of 31 patients, signet ring cells were present in 8 patients and in 23 patients, signet ring cells were absent. Among those 23 patients, 10 (43%) patients showed gene amplification by FISH. From 12 (39%) lymphnode positive cases, 7 (58%) cases showed *HER2* gene amplification.

In terms of tumor location, most of the tumors were present at distal stomach site. In 21 Patients tumors, were located at distal stomach site among those 10 patients showed tumors at antrum site and 11 patients showed at pylorus site. Out of total 21 cases, 6 cases (60%) had tumors localized in the antrum showed *HER2* positivity while at pylorus site only 27% (3/11) were showed *HER2* positivity (Table:3).

Table No. 3: *HER2* gene correlation with distal tumor location

Distal tumor location	***HER2* Positive by FISH**	***HER2* Negative by FISH**
Antrum (n=10)	6 (60%)	4 (40%)
Pylorus (n=11)	3 (27%)	8 (73%)

Out of 31 cases, 2 cases showed highly amplified *HER2* gene. Among that one case showed high *HER2* gene amplification in a 30 years female patient. The distal tumor location was at antrum and tumor differentiation was poorly differentiated adenocarcinoma. The patient had liver metastasis at the time of the diagnosis. Second case was 62 years male patient who showed cluster formation of amplified *HER2* gene. Tumor was moderately differentiated adenocarcinoma. The patient was bone metastasized at last follow-up.

DISCUSSION

In India Gastric cancer is the fifth most common cause of cancer related deaths[15]. Around the world, gastric cancer has been managed with different treatment plans. For nonmetastatic disease, surgery is the mainstay treatment. However, in clinical practice majority of patients have advanced or metastatic disease, where chemotherapy is used as a standard treatment for palliative care and survival. In recent years chemotherapy has emerged along with several molecular targeting agents for better survival.

HER2 expression in gastric carcinoma is known for >30 years[16]. Efficacy of trastuzumab in gastric cancer has been investigated with large multicentric study trials (ToGA trial)[17]. *HER2* positivity in gastric carcinoma ranges from as low as 4% to as high as 53%. Sekaran et al. from Hyderabad (India) have reported *HER2* positivity of 44.2%[18]. Another study from India reported *HER2* positivity of 35.9 %[19]. Similarly, in present study *HER2* gene amplification was 45%, which correlates with the range previously reported. According to Tanner M et al. and Barros-Silva JD et al. the range of *HER2* amplification is in between 9.3% to 22.6%, which is remarkably low as compare to present study[20, 21]. This may be explained as geographic difference.

In present study, higher *HER2* gene amplification was seen in older age patients. However, the correlation was not statistically significant, which was consistent with earlier reports[22]. Inconsistent results have been reported for tumor location and *HER2* expression. Many authors did not report any significant association between site of tumor and *HER2* positivity, on the contrary Laboissiere et al. reported a significant association of *HER2* positivity with distal tumor site[23]. In the present study, we found *HER2* positivity at distal tumor site as compared to proximal site; however, association was not statistically significant. Strong association of *HER2* positivity with tumor differentiation was observed, which was statistically significant ($p \leq 0.05$). This has been consistently proven in other studies as well[24-25].

In the present study, no correlation was found between *HER2* amplification and signet ring cells, tumor location and lymphnode involvement. This could be due to a relatively lower *HER2* positivity seen in resection and biopsy specimens. Significance of *HER2* amplification has been approved for the treatment of positive gastric adenocarcinoma. Hence, Aditi R et al. described that in the diagnostic work

up; *HER2* status should now be routinely included in patients with advanced or metastatic gastric cancer[26]. Based on the results of ToGA trial, the US FDA granted the approval for trastuzumab (Herceptin, Genentech, Inc.) [27]. Indian Council of Medical Research (ICMR) suggests the use of trastuzumab as targeted therapy in *HER2* positive advanced gastric cancer only[28].

In summary, the present study focused on importance of routine clinical testing of gastric tumors for *HER2* expression in Indian patients. Also, the work emphasised on the role of FISH in solid tumors. FISH has tremendous sensitivity and specificity in detecting *HER2* gene amplification. Present study highlights the higher rate of *HER2* positivity in moderately and poorly differentiated types of adenocarcinoma which explain the bad prognosis associated with *HER2* positivity. Thus, detection of *HER2* amplification would help to identify specific subsets of the patients. *HER2* would serve as a prognostic and predictive biomarker in gastric cancers. In addition to its established clinical value for selection of the patients for trastuzumab therapy.

REFERENCES

1. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Annals of oncology. 2008 Sep; 19:1523-9.
2. Lee HE, Park KU, Yoo SB et al. Clinical significance of intratumoral HER2 heterogeneity in gastric cancer. European journal of cancer. 2013 ; 49 :1448-57
3. Moasser, Mark M. "The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis." Oncogene 2007; 45: 6469-6487.
4. Murthy SS, Sandhya DG, Ahmed F et al. Assessment of HER2/Neu status by fluorescence in situ hybridization in immunohistochemistry-equivocal cases of invasive ductal carcinoma and aberrant signal patterns: a study at a tertiary cancer center. Indian Journal of Pathology and Microbiology. 2011; 54:532.
5. Shang AQ, Wu J, Bi F et al. Relationship between HER2 and JAK/STAT-SOCS3 signaling pathway and clinicopathological features and prognosis of ovarian cancer. Cancer biology & therapy. 2017; 18:314-22.
6. Pillai RN, Behera M, Berry LD et al. HER2 mutations in lung adenocarcinomas: a report from the Lung Cancer Mutation Consortium. Cancer. 2017; 123: 4099-105.

7. Sharifi N, Salmaninejad A, Ferdosi S et al. HER2 gene amplification in patients with prostate cancer: Evaluating a CISH-based method. Oncology letters. 2016; 12(6):4651-8.
8. Li Q, Wang D, Li J, Chen P. Clinicopathological and prognostic significance of HER-2/neu and VEGF expression in colon carcinomas. BMC cancer. 2011; 1:1-6.
9. Abrahao-Machado LF, Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. World Journal of gastroenterology. 2016; 22(19):4619.
10. Hofmann M, Stoss O, Shi D et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology. 2008; 52(7): 797-805.
11. Barok M, Tanner M, Köninki K et al. Trastuzumab-DM1 is highly effective in preclinical models of HER2-positive gastric cancer. Cancer letters. 2011; 306(2):171-9.
12. Bray F, Ferlay J, Soerjomataram I et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018; 68(6):394-424.
13. Liu X, Wang X, Wang B et al. HER2 gene amplification by fluorescence in situ hybridization (FISH) compared with immunohistochemistry (IHC) in 122 equivocal gastric cancer cases. Applied Immunohistochemistry & Molecular Morphology. 2016 ;24(7):459-64.
14. Wolff AC, Hammond ME, Allison KH et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. Archives of pathology & laboratory medicine. 2018; 142(11):1364-82.
15. Available from: http: //www.cancerindia.org.in/globocan2018-india-factsheet
16. Yokota J, Toyoshima K, Sugimura T et al. Amplification of c-erbB-2 oncogene in human adenocarcinomas in vivo. The Lancet. 1986; 327(8484):765-7.
17. Bang YJ, Van Cutsem E, Feyereislova 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.
18. Sekaran A, Kandagaddala RS, Darisetty S et al. HER2 expression in gastric cancer in Indian population—an immunohistochemistry and fluorescence in situ hybridization study. Indian Journal of Gastroenterology. 2012; 31(3):106-10.

19. Lakshmi V, Valluru VR, Madhavi J, Valluru N. Role of her 2 neu in gastric carcinoma-3 year study in a medical college hospital. Indian J Appl Res. 2014;4(11).
20. Tanner M, Hollmen M, Junttila TT et al. Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIα gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. Annals of Oncology. 2005; 16(2):273-8.
21. Barros-Silva JD, Leitao D, Afonso L et al. Association of ERBB2 gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients. British journal of cancer. 2009; 100(3):487-93.
22. Roy PS, Nyodu T, Hazarika M et al. Prevalence of HER2 expression and its correlation with clinicopathological parameters in gastric or gastroesophageal junction adenocarcinoma in north-east Indian population. Asian Pacific journal of cancer prevention: APJCP. 2019; 20(4):1139.
23. Laboissiere RS, Buzelin MA, Balabram D et al. Association between HER2 status in gastric cancer and clinicopathological features: a retrospective study using whole-tissue sections. BMC gastroenterology. 2015; 15(1):157.
24. Shan L, Ying J, Lu N. HER2 expression and relevant clinicopathological features in gastric and gastroesophageal junction adenocarcinoma in a Chinese population. Diagnostic pathology. 2013; 8(1):76.
25. Van Cutsem E, Bang YJ, Feng-Yi F et al. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. Gastric Cancer. 2015; 18(3):476-84.
26. Aditi R, Aarathi R, Pradeep R. HER2 Expression in Gastric Adenocarcinoma—a Study in a Tertiary Care Centre in South India. INDIAN J SURG. 2016; 7:18-24.
27. U. S. Food and Drug Administration. Available from: http://www.fda. gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm230418.htm. Accessed 7 Dec 2015.
28. Shrikhande SV, Sirohi B, Barreto SG et al. Indian Council of Medical Research consensus document for the management of gastric cancer. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology. 2014; 35(4):239.