Original Article

Frequency of HER-2 positive and expression pattern in gastric adenocarcinoma by immunohistochemistry

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Objective: To determine the frequency and expression of HER2/neu positivity by immunohistochemistry in gastric adenocarcinoma.

Methodology: A total of 68 cases of gastric adenocarcinoma were included and Immunohistochemical marker for HER2/neu was applied and evaluated on paraffin sections. HER2/neu overexpression according to age, gender, site and histological type was determined. HER2/neu was assessed on the membrane staining of tumor cells. It was considered positive when more than 10% of the cells stained positively in resection specimens and for biopsy specimens at least 5 cells were positive.

Results: Out of 68 cases, 42 (61.8%) were gastric cardia in origin, 16 (23.5%) of gastoesophageal junction and 10 (14.7%) of gastric fundus. 3+ HER2/neu staining in gastric cardia, gastroesophageal junction and gastric fundus was found to be 6 (14.3%), 6 (37.5%) and 2 (20%), respectively. 36 (52.9%) cases were intestinal type and 32 (47.1%) were signet ring type and 3+ HER2/neu staining was 12 (33.3%), and 2 (6.3%), respectively. There were 3+ positive in 14 (20.6%) cases, 0, 1+ negative in 38 (55.9%) cases and 2+ equivocal in 16 (23.5%).

Conclusion: HER2/neu overexpression can be determined in gastric adenocarcinoma by using immunohistochemistry. Approximately 21% cases showed overexpression in our study. Larger studies are required for more definitive results to accurately determine proportion of patients who may benefit from trastuzumab therapy. (Rawal Med J 202;45:537-540).

Keywords: Gastric adenocarcinoma, HER2/neu, Immunohistochemistry.

INTRODUCTION

Gastric carcinoma is a frequent malignancy all over the world. It is the fourth most frequent cancer globally responsible for the second highest cancer related mortality rate by approximately 70,000 per year. Ten year data analysis at Armed Forces Institute of Pathology in Pakistan revealed that stomach cancer was the 9th commonest cancer in males.[1] Data from Karachi Cancer Registry revealed that gastric cancer is on the rise in Pakistan and both genders showed an increase in incidence from 1995-2002.[2]

HER2/neu protein functions as a transmembrane tyrosine kinase receptor and is a member of the epidermal growth factor receptors (EGFRs) family. Overexpression and amplification of HER2/neu has been detected in 10-34% of invasive breast cancers and is predictive of aggressive behavior with poor response to routine chemotherapy.[3] Overexpression and amplification of HER2 has also been documented in a number of other cancers such as colon, ovarian and bladder cancers.[4]

Similarly, overexpression and amplification has been described in 6-35% of gastric and gastro-esophageal junction (GEJ) adenocarcinomas according to different studies.[5] Several studies have shown the antitumor effect of trastuzumab in HER2/neu positive human gastric cancers. ToGA trial (Trastuzumab for gastric cancer) was the first randomized trial which provided prospective information on HER2/neu positive rates in gastric carcinoma. The study reported an overall HER2/neu positivity rate of 22.1% in 3807 patients included in the study. Patients with HER2/neu positive gastro esophageal and gastric adenocarcinomas were randomized to receive trastuzumab (Herceptin), Trastuzumab with chemotherapy (CT) including 5-fluoruracil or capecitabine and cisplatin for 6 cycles or chemotherapy alone. Better response was seen in the group given trastuzumab with chemotherapy (47.3%vs. 34.5%).[6] The objective of this study was to evaluate HER2/neu positivity in gastric carcinoma by immunohistochemistry in our patients.

METHODOLOGY

The study was carried out at Department of Pathology, Shifa International Hospital, (SIH)

Islamabad from September 2010 to January 2012. Approval of ethical committee of was taken prior to the study. A total of 68 cases of gastric adenocarcinoma diagnosed during this period on endoscopic biopsies or resections were included. Poorly fixed tissue, patients who had received chemotherapy and biopsies with very scanty viable tumor cells were excluded from the study.

Gastric adenocarcinoma was diagnosed on H/E slides. Immunohistochemistry was done by using HER2/neu antibody (Clone CB11) and manual method. In manual method, sections of 5 micron thickness are cut then deparaffinized in xylene and rehydrated. Than tissue were placed in hydrogen peroxide for 10 minutes. Slides were dipped in EDTA buffer and placed in hot oven for one hour. Slides were than washed in OBS buffer for 5 minutes.

Slides were placed in incubator and blocking antibody poured for ten minutes. After 10 minutes, blocking antibody was poured off and sections were incubated with primary antibody. Her2/neu antibody came as ready to use (concentration 6.5 mg/L) and this was directly applied on the sections. Lastly, section was covered with streptavidin-peroxidase antibody and incubated and counterstained with hematoxylin.

The slides were scored for basolateral membrane staining as recommended in standard guidelines recommended by TOGA trial.[6] Strong membrane staining of more than 10% tumor cells in resection specimens and more than 5 cells in biopsies was considered positive (3+) Weak staining was considered borderline/equivocal (2+) and barely perceptible staining or no staining was considered negative (1+/0).

Statistical Analysis: The data were analyzed by using SPSS version 20. A Chi-Square test was performed, while $P<0.05$ was considered statistically significant.

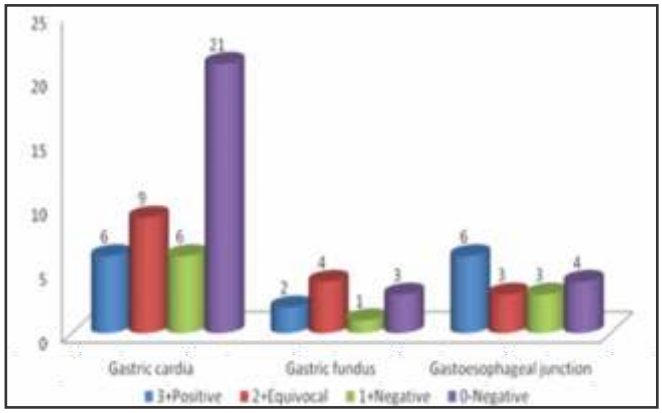
RESULTS

A total of 68 cases with age range from 19-80 years were included in the study. A total of 49 (72.1%) patients were males and 19 (27.9%) were females. Male to female ratio was 2.6:1

(p=0.01). Most frequent age decade was fifth decade (30.9%) followed by fourth decade (26.5%). Age was compared with staining (p=0.062). Endoscopic specimens were 62 and gastric resection specimens were 6. The mean age of patients was 53.3±13 years.

Most frequent site for carcinoma was gastric cardia comprising 42 (61.8%) cases; followed by 16 (23.5%) from gastro esophageal junction and 10 (14.7%) from gastric fundus (p=0.33) (Fig. 1). Thirty-six (52.9%) cases were of intestinal type of adenocarcinoma and 32 (47.1%) were of diffuse (signet ring type). Out of 68 cases HER2/neu was positive in 14 (20.6%) cases, negative in 38 (55.9%) cases and equivocal in 16 (23.5%) cases. Out of the 42 gastric cardia cases, positive cases were 6 (14.3%), equivocal cases were 9 (21.4%) and negative cases were 27 (64.3 %). Gastro esophageal junction was second most frequent site with 16 cases out of which positive cases were 6 (37.5%) , equivocal cases were 3 (18.8%) and negative cases were 7 (43.8%). Gastric fundus was the site for 10 cases with only 2(20%) positive cases, 4 equivocal cases (40%) and 4 negative cases (40%).

Fig. 1. HER2/neu Staining Score according to site of gastric carcinoma.



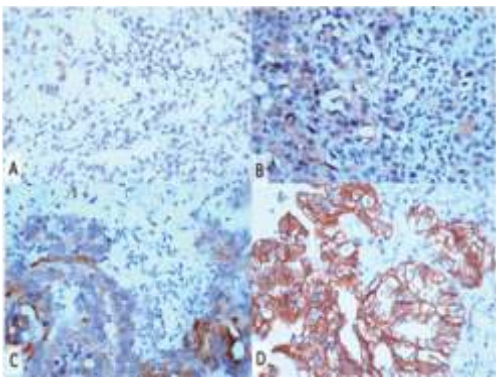


Fig. 2. HER2/neu Staining Score. A. 0 Score (Negative), B 1+ Score (Negative), C2+ Score Equlvocal D: 3+ Score (Positive).

Out of the 36 intestinal types of adenocarcinomas, positive cases were 12(33.3%), equivocal cases were 9 (25%) and negative cases were 15 (41.7%). In 32 cases of signet ring type, positive cases were 2 (6.3%), equivocal cases were 7 (21.9%) and negative cases were 23 (71.9%). HER2/neu staining of a positive case in intestinal type of carcinoma (Fig. 2). On correlation of histological type with HER2/neu positivity p-value was 0.003. There was no significant correlation between HER2/neu overexpression and gender, age or site of carcinoma.

DISCUSSION

The mean age of gastric carcinoma patients in our study was 53.43±13.02 years. Other studies revealed comparatively higher median ages. In one study by Waymann et al mean age was 70 years for men and 74 years for females.[7] The most frequent decade affected in our study was the fifth decade (31.5%) followed by fourth decade (25.9%). However, age does not play any role in HER2/neu results in gastric adenocarcinoma. This was shown in study by Tanner et al from Finland which included 131 patients of gastric adenocarcinoma and 100 patients of gastro esophageal junction cancer.[8]

Globally, gastric adenocarcinoma is more common in males as compared to females. Similar trend was seen in our study in which 49 (72.1%) patients were males and 19 (25.9%) were females. The results were comparable to other studies such as those from UK & Turkey.[7,9] Three studies showed that HER2 overexpression was more in gastroesophageal cancers as compared to gastric cancer. Gravalos showed 25% vs 9.5 % (p=0.01) HER2/neu overexpression in gastro-esophageal junction and gastric cancer, respectively.[10] Tanner et al also found high rate of HER2/neu overexpression in gastro-esophageal junction cancers as compared to gastric cancers (24% vs 12%).[8]

There is a high correlation between HER2/neu expression and histological type. This was first seen in 1990. One western study reported a significantly higher rate of HER2/neu over expression in intestinal type than the diffuse type (16% vs 7%).[11] Similarly, in a Korean study also intestinal type exhibited higher rate of amplification than the diffuse type ($p<0.05$).[12] In the ToGA trial, HER2/neu positivity differed significantly by histological subtype (intestinal - 34%, diffuse - 6%, mixed 20%).[6] Our study also showed higher frequency of positivity in intestinal type gastric adenocarcinoma than the diffuse/signet ring type (33.3% and 6.3%), respectively.

This was also comparable with the other studies by Tanner et al, Gravalos et al, and Lordick et al where HER 2/neu overexpression in intestinal vs diffuse type was found to be 21.5% vs 2% ,16 % vs 7% and 34 % vs 6 % respectively.[8,10,13] Our study showed overall HER2/neu overexpression in 20.6% cases. Her2 neu overexpression seen in 38% cases.[14] Another study from Pakistan showed a frequency of 34.5% of HER2/neu overexpression.[15] A study from India reported a positivity of 23%.[16] In breast carcinoma, concordance studies have been done to standardize assessment of HER2/neu amplification and overexpression by FISH and IHC. Concordance between the two methods is found to be 73% -98%.[17] Lemoine et al analyzed 40 cases and found HER 2/neu protein overexpression in 26% cases and gene amplification in 13% cases.[18] However, recent studies have shown higher concordance between HER2 overexpression by IHC and gene amplification in situ hybridization techniques.[19,20]

CONCLUSION

HER2/neu overexpression can be determined in gastric adenocarcinoma by using immunohistochemistry. Intestinal type of adenocarcinoma shows more frequent HER2/neu overexpression.

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