HER-2/NEU EXPRESSION IN STOMACH AND GASTRO-OESOPHAGEAL JUNCTION CARCINOMA IN ENDOSCOPIC BIOPSY SPECIMENS

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ABSTRACT

BACKGROUND

Prognosis of patients with advanced inoperable carcinoma of stomach and gastro-oesophageal junction remains dismal with palliative chemotherapy. In spite of extensive efforts, the five-year survival rate of these patients worldwide is 20%. The main problem with chemotherapy is its toxicity and thus the management of these patients is very complex. These patients also have associated comorbidities. Therefore, there is a need to understand the molecular biology of these malignancies and develop personalised, specialised and molecular targeted therapy. Hence, we planned this study to evaluate the expression of HER-2/neu in carcinoma of stomach and gastro-oesophageal junction.

MATERIALS AND METHODS

This was an observational study from January 2010 to December 2016 wherein all cases of carcinoma of stomach and gastro-oesophageal junction were retrieved and reviewed. Immunohistochemistry for HER-2/neu protein was done. Statistical analysis was done using SPSS 10.0 windows student version {SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412}.

RESULTS

The age of patients varied from 34 to 72 years and mean age was 58.5 years. The common clinical presentation was dyspepsia and regurgitation (82.4%). Of total 85 cases, the grade of HER-2/neu expression was as follows: 0 in 65 (76.47 %) cases, 1+ in 2 (2.3%) cases, 2+ (equivocal) in 4 (4.70 %) cases and 3+ in 14 (16.47 %) cases.

CONCLUSION

Assessment of HER-2/neu gene amplification by FISH technique and HER-2/neu protein overexpression by immunohistochemistry should be done in all patients with gastric and gastro-oesophageal carcinoma especially inoperable cases so that these patients are treated with molecularly targeted therapy.

KEYWORDS

Carcinoma, Stomach, Gastro-oesophageal Junction, HER-2/neu and Immunohistochemistry.

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BACKGROUND

Stomach carcinoma is the fourth most common cancer in incidence and second most common cause of cancer deaths.[1] The annual incidence of carcinoma of stomach and gastro-oesophageal junction is 1.4 million cases, and annual deaths because of these malignancies are 1.1 million. Therefore, carcinoma of stomach and gastro-oesophageal junction poses a major global health problem.[2] In India, the incidence of gastric carcinoma is higher in the southern and north-eastern states with Mizoram recording an age-adjusted rate of 50.6 and 23.3 for men and women respectively.[3,4] A recent assessment of 556,400 deaths due to cancer in India in 2010 based on a nationally representative survey found that stomach cancer with a mortality rate of 12.6% is the second most common fatal cancer.[5]

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The incidence of carcinoma stomach has declined in the recent past whereas the incidence of carcinoma gastro-oesophageal junction has risen dramatically.[2] Despite advances in the treatment and management of carcinoma stomach, 5-year survival rate remains around 20% in most parts of the world except Japan where 5-year survival rate is around 60% due to screening programmes, staging systems and treatment.[6] The poor survival rates in carcinoma of the stomach and gastro-oesophageal junction are because most of the patients present in advanced stages at the time of diagnosis. These patients present in advanced stage because their clinical presentation is initially nonspecific and by the time they are diagnosed, they present with local and metastatic spread. If the disease can be diagnosed at earlier stages through effective screening programmes and rapid surgical resection be performed as in Japan, the five-year survival can be raised to approximately 60%.[1] Chemotherapy is the mainstay of treatment of patients with inoperable gastrointestinal cancer. Treatment of inoperable carcinoma of stomach and gastro-oesophageal junction is complex involving surgery combined with perioperative radiotherapy and/or chemotherapy. Chemotherapy achieves a significantly better overall survival rate as compressed to best supportive care alone.[7] Conventional chemotherapeutic agents which are used in the inoperable carcinoma stomach are used in combination

of three drugs [5-fluorouracil, cisplatin and epirubicin]. This is the most effective combination resulting in median survival of 8 – 11.2 months. No consensus exists as for as the modalities are concerned.[8,9] In spite of extensive efforts in the management and treatment of these patients, the prognosis of these patients with palliative chemotherapy remains dismal.[9] Toxicity is one of the main problem associated with aggressive chemotherapy especially in elderly patients with many comorbidities. Therefore, efforts are being made to investigate molecular biology of this disease and find out molecular targets.[10] The molecular targets of carcinoma of stomach and gastro-oesophageal junction are epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), mammalian targets of rapamycin (mTOR), Cyclooxygenase-2 (Cox-2) and other factors involved in the cell cycle.[7] Therefore, we planned this study to evaluate the expression of HER-2/neu in endoscopic biopsies of carcinoma of stomach and gastro-oesophageal junction on formalin fixed paraffin embedded blocks.

MATERIALS AND METHODS

This was an observational study. All cases of carcinoma of stomach and gastro-oesophageal junction signed out in the Department of Pathology, Government Medical College, Jammu from January 2010 to December 2016 were retrieved from surgical Pathology files and consult files. In total, 130 cases were identified over a period of six years. Haematoxylin and eosin stained sections of 5 micrometre thickness were re-examined in all cases to confirm the diagnosis. Immunohistochemistry for HER-2/neu protein was done in 85 cases. In 45 cases, the material in paraffin blocks was inadequate. Clinical features and followup data was obtained from consult files and referring surgeons.

Immunohistochemistry

Immunohistochemistry for HER-2/neu was done with the monoclonal antibodies obtained from M/s Novocastra Laboratories, Newcastle upon Tyne, UK. The LSAB kit was obtained from M/s Dako Patts, Denmark. It was done on one representative cross-sectional slide per tumour, displaying a maximum of tumour mass. We used Hofmann's criteria for immunohistochemical expression of HER-2. Hoffmann's criteria is based on the percentage of cells with membrane-like staining.[11, 12] Grades are classified as follows:

Grade 0: no reaction or <10% reactivity in tumour cells; grade 1+: weak reactivity >10% of the tumour cells; with reactivity only in part of the membrane; grade 2+: moderate reactivity in >10% of the cells, with staining across the lateral and basolateral membrane; grade 3+: strong reactivity with intense staining of the lateral and basolateral membrane in >10% of the cells. We used breast cancer, tissue HER-2 positive as external positive control. Negative control was obtained by omitting the primary antibody. We considered strong and moderate (2+/3+) intensity staining as positive, and cases with low intensity and without reactivity as negative.

Statistical analysis was done using SPSS 10.0 windows student version {SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412}.

RESULTS

The age of patients varied from 34 to 72 years and mean age was 58.5 years. The common clinical presentation was dysplasia and regurgitation (82.4%). Of total 85 cases, the grade of HER-2/neu expression was as follows: : 0 in 65 (76.47 %) cases, 1+ in 2 (2.3%) cases, 2+ (equivocal) in 4 (4.70 %) cases and 3+ in 14 (16.47 %) cases.

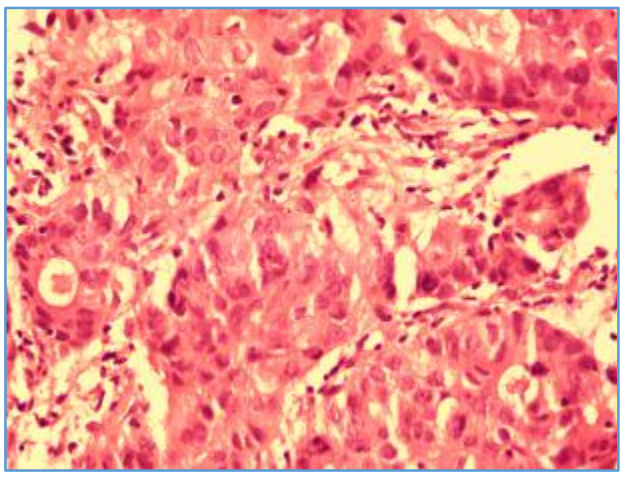

Figure 1. Microphotograph of a Case Adenocarcinoma [H& E, 400x]

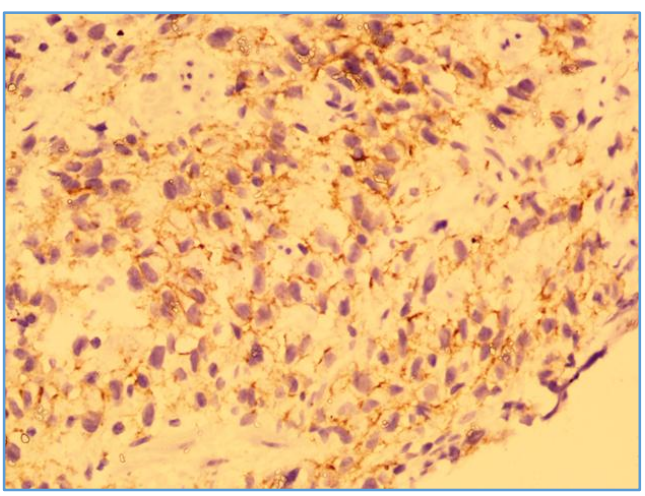

Figure 2. Immunohistochemistry for HER-2/neu in a Case of Carcinoma Stomach showing Grade 2 Positivity

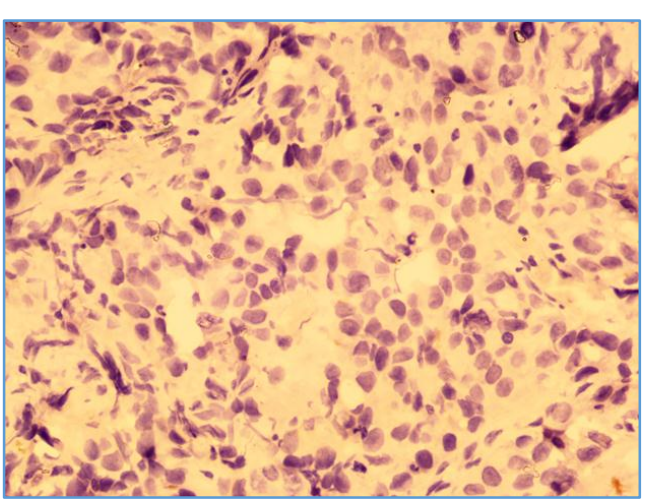

Figure 3. Immunohistochemistry in a Case of Carcinoma Stomach showing Negative Staining

DISCUSSION

HER-2 is a receptor tyrosine kinase that belongs to Group I of 20 families of receptor tyrosine kinases.[13] Other members of this group are HER-1 (erb-B1), HER-3 (erb-B3) and HER-4 (erb-B4). These tyrosine kinases are encoded by gene erb-B located on chromosome 17q21[14] {erb-B1, erb-B2, erb-B3 & erb-B4}. We know that receptor tyrosine kinases are transmembrane proteins with an extracellular ligand binding domain and a cytoplasmic domain. Receptor tyrosine kinase is activated transiently by binding of a specific growth factor to the extracellular domain, an event that induces homo- or heterodimerisation.[15] This activation leads to downstream signalling via a number of pathways including RAS, PI3K, etc. This results in cellular proliferation and survival. Oncogenic changes in these receptors may be because of mutations, gene amplification or gene rearrangement leading to constitutive growth factor-independent tyrosine kinase activity. Hence, oncogenic tyrosine kinase receptors deliver continuous signal to the cell for cellular proliferation,[16] inhibition of apoptosis[17] and promote angiogenesis.[18] HER-1 or EGFR is overexpressed in a subset of adenocarcinoma lung and mechanism involved is point mutation of erb-B1 gene. Molecularly targeted therapy is available for this subset of adenocarcinoma, lung.[19] HER-2 is also overexpressed in many carcinomas of various other organs like breast, colon, endometrium, cervix, ovary and urinary bladder which are characteristically very aggressive.[19] For the first time, HER-2 overexpression was described in carcinoma breast[20] and mechanism underlying this overexpression is amplification of erb-B2 gene. Anti-HER-2 therapy is well established in treatment of carcinoma breast[21] and this has assumed more significance with molecular classification of carcinoma breast.

HER-2/neu expression in our study is 21.17%. Our results are very similar to those found in most American and European studies, where the prevalence rates of HER-2/neu expression are from 10 % to 22.8 %.[22, 23, 24, 10, 25, 26] Most studies in Asia have reported HER-2/ neu expression prevalence of 11.7 to 15.74 %.[22, 23, 24, 10, 25, 26] In our study, the expression of HER-2/neu is 21.17 %. In a similar study in India by Dewan et al[27] from Army Hospital Research and Referral Centre, Delhi Cantt, the prevalence of HER-2/neu expression was 17.0%. So our results are almost comparable with this study. In the famous TOGA trial,[28] HER-2/neu expression was 22.1% which is comparable with our study. TOGA trial was a phase III, randomised controlled multicentre study designed to test the efficacy and safety of an anti-HER-2 drug (Trastuzumab) along with conventional chemotherapy for treatment of advanced gastric cancer. This trial showed that patients with HER-2/neu expressing tumours had a survival of 16 months when their chemotherapy was combined with trastuzumab as compared to 11.5 months in patients who were given conventional chemotherapy only.

Our study has limitations that it was done on limited material of endoscopic biopsies. Another limitation is that we did not perform FISH technique to confirm HER-2/neu amplification in equivocal cases (2 +). In these equivocal cases when evaluated by FISH technique, the positivity rate changes. This evaluation of HER-2/neu gene amplification by FISH is important because only those cases that are grade 3+ and 2+ by immunohistochemistry, that are positive for FISH amplification present evidence of more benefit of adding trastuzumab to the chemotherapy regimen.[28, 24, 6, 27] With advent of molecular targeted therapy, we need to determine HER-2/neu expression accurately in order to determine which patients are going to benefit from this drug.

CONCLUSION

Assessment of HER-2/neu gene amplification by FISH technique and HER-2/neu oncoprotein overexpression by immunohistochemistry should be done in all patients with gastric and gastro-oesophageal carcinoma so that these patients can be benefitted from molecularly targeted therapy i.e. Trastuzumab group of drugs. Technique of Immunohistochemistry for HER-2/neu and its interpretation in carcinoma stomach and gastro-oesophageal junction has now been standardised.

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