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A study of Her2/neu expression and its correlation with the histological type of gastric carcinoma

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ABSTRACT

Gastric carcinoma is a leading cause of cancer-related deaths globally, has necessitated the search for newer therapeutic regimens one of which is targeted therapy utilizing Her2neu expression. Studies have shown that the levels of Her2 protein predict the response of gastric carcinoma to Trastuzumab. This study has been done to determine Her2neu expression in gastric carcinomas and correlate it with the histological type. Immunohistochemistry for Her2neu expression was carried out on archival material from 47 cases of gastric carcinoma obtained from the Department of Pathology of Saveetha Medical College. Scoring was done based on the study by Hofmann *et al*. The histological type was classified using Lauren's classification and a correlation between the type and Her2neu expression was done. Positive Her2neu expression (3+) was seen in 8 cases (17%) of gastric carcinoma. Out of these 6 were of the intestinal type and 2 were diffuse. Well-differentiated carcinomas had a higher positivity for Her2neu as compared to moderately and poorly differentiated tumours. Her2neu expression in a relatively higher proportion of cases of gastric carcinoma would prompt routine testing for Her2neu expression in all cases. As the results of Trastuzumab therapy in Her2neu positive gastric carcinoma have been shown to be encouraging, it would be advisable to routinely test all cases of gastric carcinoma for Her2neu expression to use the targeted therapy effectively.

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INTRODUCTION

Gastric carcinoma is the third leading cause of cancer mortality in the world and the second most common cause of cancer deaths in the Indian population (Gupta P *et al.,* 2016). Moreover, according to the National Cancer Registry Program of the Indian Council of Medical Research (ICMR), the stomach is the leading site of cancer (9.1%) in Chennai, fourth leading site (6.4%) in Bangalore, and fifth problem is compounded by the fact that in India the patients present at a much later stage of the disease leading to increased morbidity and mortality (Mohandas KM, Jagannath P, 2000). In these (5.4%) in Dibrugarh (Nandi A *et al.,* 2014). The

cases, the treatment is at best palliative with chem- otherapy with multiple drugs. Even with these, the median survival has been reported to be less than 1 year (Gupta P *et al.,* 2016). This bleak scenario has prompted the necessity of finding newer mo- dalities of treatment. Extensive research done on the molecular mechanisms involved in the patho- genesis of gastric adenocarcinoma has revealedthe presence of tissue biomarkers for which tar- geted therapy with monoclonal antibodies could be a treatment option. One such biomarker is Hu- man Epidermal Growth Factor Receptor2 (HER2/neu) which has been extensively studied in breast carcinoma. The HER2/neuis a transmem- brane protein with tyrosine kinase activity, related to the epidermal growth factor receptor c-erbB-2 and is involved in normal cell proliferation and tis- sue growth (Abrahao-Machado LF, Scapulatempo-

Neto C, 2016). Its overexpression has been recognized as a frequent molecular abnormality in gastric adenocarcinoma and the rate of positivity of HER2/neu in this condition is estimated to be between 7 to 34% (Gupta P *et al.,* 2016; Abrahao-Machado LF, Scapulatempo-Neto C, 2016). In gastric adenocarcinoma, HER2/neu overexpression has been correlated with a poorer outcome and a more aggressive nature of the disease well as with shorter survival (X.L. Zhang *et al.,* 2009; H. Allgayer *et al.,* 2000; D.I. Park *et al.,* 2006). Trastuzumab is a monoclonal antibody which targets HER2/neu explicitly by directly binding to the extracellular domain of the receptor and inhibits HER2/neu mediated signalling. In the pathbreaking ToGA (Trastuzumab for gastric cancer) trial in which 24 countries participated, a humanized monoclonal antibody against HER2, Trastuzumab (Herceptin), was given in combination with chemotherapy (capecitabine or 5-fluorouracil and cisplatin) to patients with gastric adenocarcinoma and gastroesophageal junction cancer. It was found that this could prolong overall survival and progression-free survival and increase the response rate in HER2 positive advanced gastric carcinoma (Gupta P *et al.,* 2016). Following this, on October 20, 2010, the Food and Drug Administration (FDA) approved trastuzumab in combination with cisplatin and a fluoropyrimidine (either capecitabine or 5-fluorouracil) to be used for the treatment of patients with HER2-overexpressing metastatic gastric or junction adenocarcinoma. It is now recommended in many countries that all patients with gastric adenocarcinoma should routinely be tested for the HER2 status at the initial diagnosis (Van Cutsem E *et al.,* 2009). Several studies have been done on Her2/neu positivity in gastric carcinoma in the West along with the correlation of clinic-pathological characteristics. However, only a few such studies are available in the Indian population. This study was conducted to evaluate the expression of Her2/neu in cases of gastric carcinoma diagnosed at a tertiary care centre in Chennai and to correlate this with the histological type, grade and stage of a tumour.

MATERIALS AND METHODS

The study was retrospective and was done at the Department of Pathology, Saveetha Medical College, Chennai, after obtaining the necessary approval from the Institutional Review Board. The case records, Haematoxylin & Eosin (H & E) stained slides and paraffin blocks from all cases of gastric adenocarcinoma diagnosed over a two year period were accessed (January 2015 to December 2016). There was a total of 47 cases of gastric adenocarcinoma, out of which 32 were gastric biopsies and 15 were partial gastrectomy specimens. The demographic and surgical specimen details including disease stage of the cases were obtained from the case records and the histopathology records in the institution.

The H&E slides were reviewed and the histological features were noted. The gastric adenocarcinomas were classified as intestinal/ diffuse/ mixed according to the Lauren classification and grading of a tumour was done as well/moderate or poorly differentiated. According to Lauren's classification, intestinal types of gastric adenocarcinomas form recognisable glands that range from well differentiated to moderately differentiated tumours, sometimes with poorly differentiated tumour at the advancing margin. Diffuse carcinomas consist of poorly cohesive cells diffusely infiltrating the gastric wall with little or no gland formation. The cells usually appear round and small, either arranged as single cells or clustered. Well-differentiated adenocarcinomas are those with well-formed glands, often resembling metaplastic intestinal epithelium. Moderately differentiated tumours are intermediate between well differentiated and poorly differentiated. Poorly differentiated adenocarcinomas are composed of highly irregular glands that are recognized with difficulty, or single cells that remain isolated or are arranged in small or large clusters with mucin secretions or acinar structures. Staging of a tumour was done based on the American Joint Committee on Cancer (AJCC) a tumour/node/metastasis (TNM) classification and staging system for gastric cancer (Washington K 2010) Appropriate blocks were selected for immunohistochemistry (IHC) for Her2/neu staining. 4 µm thick tissue sections were taken on slides coated with Poly L Lysine. The primary antibody used was HER2 (Anti-Human c-erB-2 oncoprotein) (Dako Hercep Test TM) and the secondary antibody kit was Novolink polymer (Leica Biosystems). Positive and negative controls were used during the staining procedure. HER2/neuscoring (Table 1) was done using the semi-quantitative criteria proposed by Hofmann *et al.,* 2008. Different criteria were used for biopsy and resected specimens. Expression of HER2/neu was graded from a score of 0 to 3.

RESULTS

There was a total of 47 cases of gastric adenocarcinoma out of which 15 (31.9%) were partial gastrectomy specimens and the remaining 32 (68.1%) were gastric biopsies. A 3+ Her2 score was considered positive and score values of 2+, 1+, and 0 were considered negative. HER2/neu done by IHC was strongly positive in 8 cases (17%, 3+) and negative in 39 cases (38 were score 0 and one case with score 1+). When HER2/neu positivity was analyzed according to the type of specimen, a higher

Table 1: Interpretation of HER2/NEUImmuno-histochemical staining in Gastric and Gastroesophageal Junction Carcinoma (GGEAC)

Score	Interpretation	Criteria for GGEAC Biopsy specimens	Criteria for GGEAC Resected specimens
0	Negative	No staining or membrane staining in any of the tumour cells.	No staining or membrane staining in <10% of tumour cells
1+	Negative	Cluster(s) of at least 5 cohesive tumour cells with weak complete, basolateral, or lateral membrane staining, irrespective of tumour volume percentage	Weak complete, basolateral, or lateral membrane staining in ≥10% of tumour cells
2+	Equivocal	Cluster(s) of at least 5 cohesive tumour cells with moderate complete, basolateral, or lateral membrane staining, irrespective of tumour volume percentage	Moderate complete, basolateral, or lateral membrane staining in ≥10% of tumour cells
3+	Positive	Cluster(s) of at least 5 cohesive tumour cells with strong complete, basolateral, or lateral membrane staining, irrespective of tumour volume percentage	Strong complete, basolateral, or lateral membrane staining in ≥10% of tumour cells

rate of HER2 positivity was seen in small biopsies compared to gastrectomy specimens. Out of 32 small biopsies, 6 (18.75%) showed HER2/neu over expression and two out of 15 (13.33%) respected specimens showed HER2/neupositivity. Out of the 8 HER2/neupositive cases, 6 cases (75%) were of the intestinal type and 2 cases (25%) were of the diffuse type (Figure 1a-d). Out of the 39 HER2/neu negative cases, 21 were of the intestinal type, 15 were diffuse and 3 were of the mixed type. (Figure 1e and f)

4 cases (50%) of well differentiated, 3 cases (37.5%) of moderately differentiated and 1 case of (12.5%) poorly differentiated gastric adenocarci- noma showed strong HER2/neu positivity. Both the respected tumours (25% of positive cases) which were HER2/neu positive (3+), were in pT3 and pN3 stages. (T3 -Tumor penetrates subserosal connective tissue without invasion of the visceral peritoneum or adjacent structures. N3 - Metastasis in seven or more regional lymph nodes). Statistical analysis was done using SPSS version 22.0 soft- ware. Nominal categorical data between the groups were compared using the Chi-square test. For all statistical tests, $p<0.05$ was taken to indi- cate a significant difference.

DISCUSSION

Human epidermal growth factor receptor 2 (HER2/neu) is a 185-kDa transmembrane tyrosine kinase receptor and its overexpression following gene amplification has been observed in many solid tumours (Chao He *et al.,* 2013). Worldwide prevalence rates of HER2 expression in gastric adenocarcinomas range from 7% to 34%. In a few studies conducted in the South Indian population, the expression was higher, ranging from 35.89% to 44.2% (Lakshmi V *et al.,* 2014; Sekaran A *et al.,* 2012). In the present study too, the findings were in accordance with that found in world literature (17%). This relatively high positivity rate in the In- dian population too is further vindication for rou- tine testing for this biomarker in cases of gastric adenocarcinoma.

In our study, HER2/neu overexpression was found to be more in gastric biopsies (18.75%) than in tissue sections obtained from respected specimens (13.33%). The studies by Gupta et al. and Indu Rajagopal *et al*. also concluded that a number of gastric biopsies (31.8% and 30%) have HER2/neu overexpression compared to gastrectomy specimens (Indu Rajagopal *et al.,* 2015). Several explanations have been given for this finding. One is that the higher rates of HER2/neu positivity in biopsies may be due to the larger sample size of biopsies (n=32) when compared to gastrectomy specimens (n=15) in most studies. Another reason that has been suggested is better and quicker fixation of biopsy specimens (Josef Rüschoff *et al.,* 2012). Davidson and Pai have postulated that a higher rate of positivity in biopsies is due to better antigen preservation in biopsies as they have shorter cold ischaemic time as a result of quicker fixation. Cold ischemic time is the time between tissue removal and the initiation of formalin permeation of the tissue and is an important pre-analytic variable (Davidson JM, Pai RK 2013).

While correlating the histological type of gastric adenocarcinoma with Her2/neu positivity, it was found that the intestinal type showed a stronger association (75%) with Her2/neu positivity when compared with the diffuse type (25%). This was, however, not statistically significant in this study ($p = 0.670$). Other studies by Gupta *et al*., Sutapa *et al.* and De Carli *et al*. have also had similar findings.

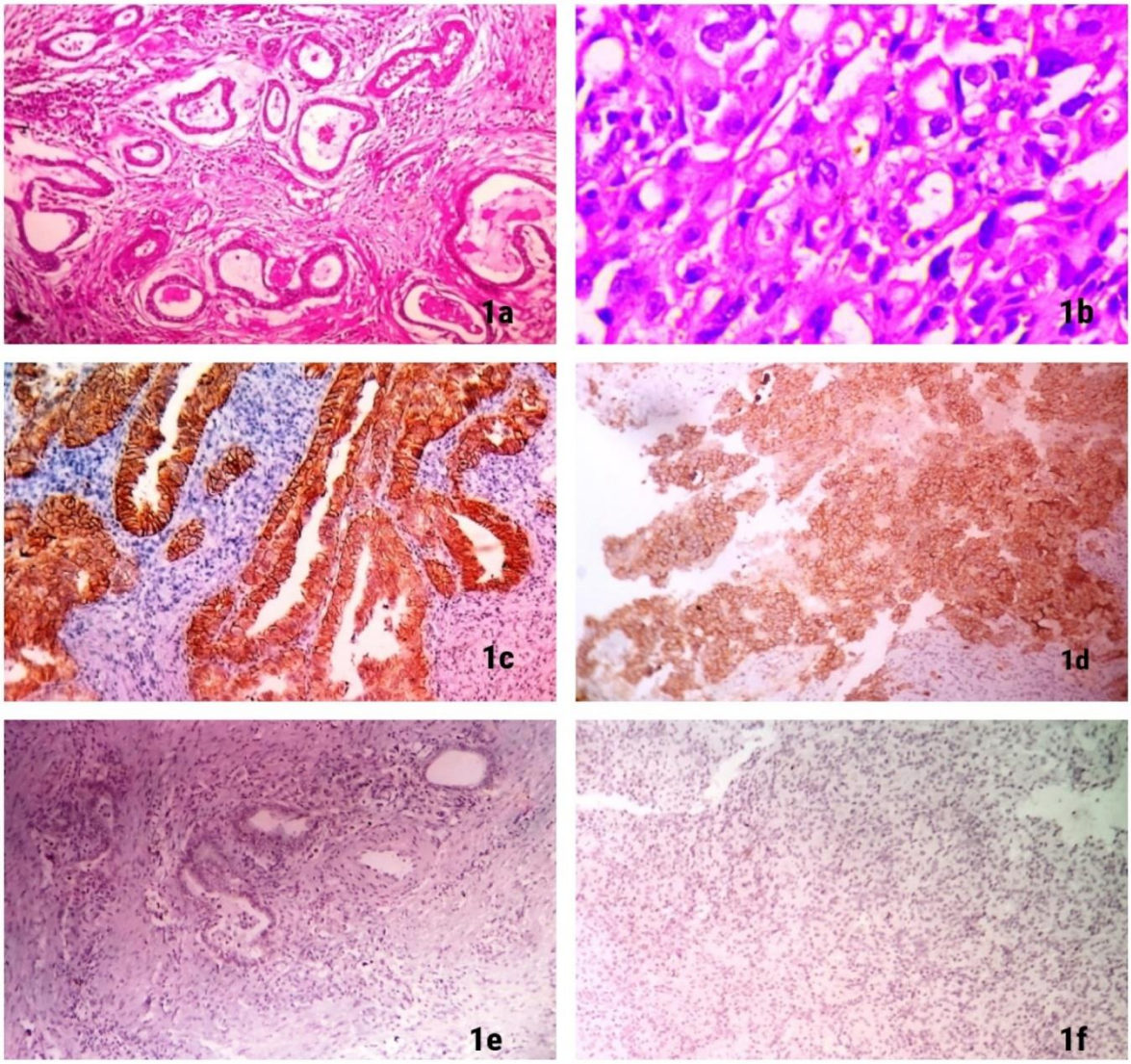

Figure 1: HER2/neu score in intestinal and diffuse types of gastric adenocarcinoma 1a. Intestinal type of gastric adenocarcinoma. H&E 100X.1b. Diffuse type of gastric adenocarcinoma. H&E 400X.1c. HER2 positivity (3+) in intestinal type of gastric adenocarcinoma IH

In the study by Gupta et al this finding was statistically significant (p= 0.005) Prevalence in the intestinal type ranges from 6.1% to 36.8%, and in the diffuse type from 0.7% to 13.43% in these studies (Sutapa Halder *et al.,* 2017; De Carli DM *et al.,* 2015). This phenomenon, wherein a tumour Characteristics correlate with Her2/neu positivity has been observed in breast carcinomas. Invasive ductal carcinomas of the breast have a higher percentage of expression of Her2/neu than lobular carcinoma. The suggestion that has been made is that this was due to loss of E-cadherin gene function in lobular carcinoma. Diffuse carcinomas of the stomach too, show loss of E-cadherin gene and an inverse relation between Her2/neu expression and E-cadherin loss has been proven in gastric tumours (Al-Nashi *et al.,* 2013; Al-Grawi et al., 2018).

Reduced Her2/neu expression with loss of differentiation in intestinal type of gastric adenocarcinomas has been found in some studies. In a study of 1463 patients which included 929 gastric adenocarcinomas, Shan et al. detected a statistically significant higher rate of Her2/neu positivity in well-differentiated and moderately differentiated gastric adenocarcinomas as compared to poorly differentiated tumours (P<0.01). The same observation was made by Lakshmi et al. (60% positive in well differentiated, 29.1%, in moderately differentiated adenocarcinoma and 20.6% in poorly differentiated adenocarcinoma) and Decarli *et al*. (25% positive in well differentiated) (Lakshmi V *et al.,* 2014). In the present study, number of well and moderately differentiated tumours had 3+ Her2/neu positivity which was statistically significant (p=0.043) However, there have been conflicting reports about this association, the reasons for which have been attributed to causes such as varying sample sizes, lower prevalence of Her2/neu positivity and the varying methods of scoring that existed prior to the establishment of the current

guidelines. Further studies using these well-defined guidelines are expected to give more clarity on this finding (Shan *et al.,* 2013).

No significant correlation was found between HER2/neu overexpression and tumour stage in the present study. The two respected tumours which showed HER2/neu (3+) positivity were both in pT3 and pN3 stages and this was not statistically significant. Studies conducted by Gupta et al., Sutapa Halder *et al.* and Shan *et al.* have also found no statistical significance in this feature (Sutapa Halder *et al.,* 2017; Shan *et al.,* 2013).

CONCLUSION

This study has shown that HER2/neu positivity is seen in a relatively significant number of cases of gastric adenocarcinoma even in the Indian population. As gastric cancer has significant morbidity and mortality – newer treatment protocols are required – beyond surgery and chemotherapy. Targeted therapy such as monoclonal antibodies against HER2/neu has become the need of the hour and hence routine testing for this biomarker is recommended. More studies involving a larger number of cases would give greater clarity on these tumours in the Indian population.

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