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Original Article

Pathology Section

Evaluation of Expression of HER2/neu in Intestinal and Diffuse Type Gastric Carcinomas by Immunohistochemistry

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ABSTRACT

Introduction: The management of gastric cancer in advanced stages, which is the second most leading cause of cancer mortality in the world, has evolved very little. Surgical resection remains the mainstay of treatment and can cure patients with early-stage cancer. The survival rate of patients with advanced resectable-gastric cancers, however, remains poor despite new treatment strategies. A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signalling cascades involved in cell differentiation, proliferation and survival.

Aim: To evaluate the expression of HER2 (Human epidermal growth factor receptor 2) by Immunohistochemistry (IHC) in intestinal and diffuse type gastric carcinomas, in and around Kanchipuram, Tamil Nadu, India.

Materials and Methods: Fifty cases (31 males, 19 females) were selected which includes 30 cases of resected gastric carcinoma specimens and 20 cases of endoscopic guided biopsies diagnosed as gastric carcinoma in the Department of Pathology, in Meenakshi Medical College and Research Institute (MAHER), Enathur, Kanchipuram, Tamil Nadu which is a teaching medical college and hospital in southern part of-India from January 2012 to July 2014 for a period of two and half years. All the cases that were received during the study period that-fulfilled the inclusion and exclusion criteria were included in the study. All cases were analysed for their expression of HER 2/Neu protein antigen by Immunohistochemical staining in this study. The scoring criteria established by an international consensus panel of oncologists and pathologists was followed. Breast carcinoma tissue positively expressing HER2 was used as the positive control. We considered only those tumours expressing 3+positivity as those expressing HER2.

Results: HER2 score was significantly higher in intestinal type gastric carcinomas. Of the total 26 cases expressing the HER2 protein, 81% (21/26) were of intestinal type Vs 19% (5/26) that of diffuse types (p=0.007). Also, no statistical difference was found between other variables like age and gender (p=0.098 and p=0.985).

Conclusion: This study assessed the expression of HER2 in gastric carcinomas and we found a statistically significant expression in intestinal type gastric carcinomas than the diffuse type gastric carcinomas. There was no significant overexpression of HER2 protein regarding the clinicopathological variables like age and gender.

Keywords: Clinicopathology, Fluorescent in situ hybridization, Histopathology, Lauren's classification

INTRODUCTION

The management of gastric cancer in advanced stages, which is the second most leading cause of cancer mortality in the world, has evolved very little [1]. Surgical resection remains the mainstay of treatment and can cure patients with early-stage cancer. The survival rate of patients with advanced resectable-gastric cancers, however, remains poor despite new treatment strategies, such as perioperative chemotherapy or adjuvant chemoradiation as a result of which new therapies are urgently needed. A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signalling cascades involved in cell differentiation, proliferation, and survival.

Biological prognostic factors which represent a crucial step to gastric cancer includes Human epidermal receptor 2 (HER2), Epidermal growth factor receptor (EGFR), Vascular Endothelial Growth Factor Receptor (VEGF), microsatellite instability, E-cadherin, DNA copy number changes, and changes in the expression of several factors like thymidilate synthase, matrix metalloproteinases, beta-catenin, mucin antigen, p53, COX-2 [2]. As they are the molecular targets either to chemotherapeutics or targeted therapies, these are not only prognostic factors, but could also be predictive of therapy like trastuzumab in HER2 positive tumors.

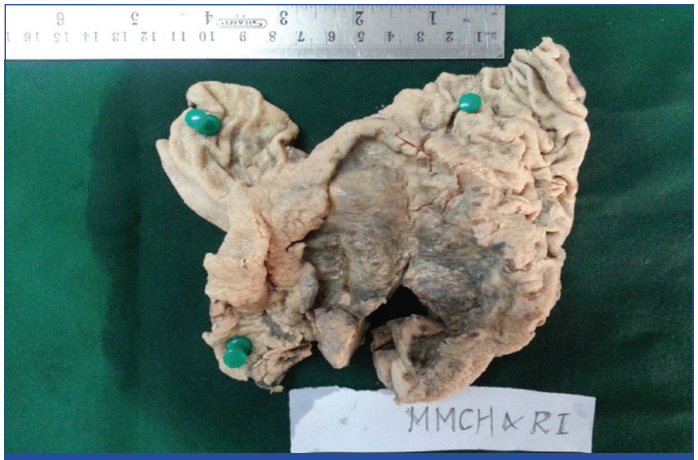
HER2 amplification and overexpression have been found to promote tumourigenesis and to be involved in pathogenesis of several human cancers like colon, bladder, ovarian, endometrial, lung, uterine cervix, head and neck, oesophageal and gastric carcinomas [3]. The gene for the HER2 protein (ErbB-2, Her2/neu or c-erbB2) is a proto-oncogene located on the chromosome 17q21 [4]. HER2 amplification is seen in 30% of human breast cancers [5]. As there are only few studies done on the expression of HER2 in gastric carcinomas in south Indian population, as compared to the number of studies on the expression of the same in breast carcinomas, in our study, we have made an attempt to analyse the expression of HER 2 in Intestinal and Diffuse type gastric carcinomas by IHC.

MATERIALS AND METHODS

The present study was a prospective study which was conducted over a period of two and a half-years from January 2012 to July 2014. The study was conducted in Meenakshi Medical College and Research Institute (MAHER), Enathur, Kanchipuram, Tamil Nadu, India, which is a teaching medical college and hospital in southern part of India. The study was approved by the institutional ethical committee. Fifty cases (31 males, 19 females) were selected which includes 30 cases of resected gastric carcinoma specimens and 20 cases of endoscopic guided biopsies diagnosed as gastric carcinoma in the Department of Pathology [Table/Fig-1].

Inclusion criteria: Gastric carcinomas diagnosed in the resected specimens and small biopsies were included in the study.

Exclusion criteria: Those samples which were seen having features other than carcinomatous features were excluded from the study.



[Table/Fig-1]: Gross picture showing gastric carcinoma with ulceration.

All the cases we received during the study period that fulfilled the inclusion and exclusion criteria were included in the study. Convenient sampling was done. The data on the age, gender and other clinical details of the patients were obtained by reviewing clinical and pathological records. Haematoxylin-eosin stained slides of the cases were evaluated and findings were noted in the prescribed data sheet. The H&E stained slides were histologically classified according to Lauren's classification [6] and WHO classification [7] and macroscopically classified according to the Borrmann's classification [Table/Fig-2] [8]. All slides were analysed for their expression of HER2 protein by IHC.

Pathological features	Numbers	Percentage (%)
Anatomical distribution of tumours		
Cardia	19	38
Fundus	4	8
Body	6	12
Antrum	15	30
Pyloric canal	6	12
Tumour distribution-by Borrman's Classification in resected specimens		
Polypoid	5	16.7
Fungiform	6	20
Ulcerated	12	40
Infiltrative	7	23.3
Tumour distribution-by-WHO Classification		
Tubular/ Papillary	23	46
Mucinous	12	24
Signet ring cell type	10	20
Undifferentiated	5	10
Tumour distribution-by Lauren's Histological Classification		
Intestinal type	28	56
Diffuse type	22	44
Differentiation in intestinal type CA in resected specimens		
Well differentiated	5	29.5
Moderately differentiated	8	47
Poorly differentiated	4	23.5

[Table/Fig-2]: Pathological features of tumours of the studied cases.

Haematoxylin-eosin slides of all the tissues were evaluated, and for each of the cases, the best paraffin blocks, having the highest tumour content and a negligible amount of necrosis and haemorrhage were chosen in order to prevent artefact staining. These formalin fixed, paraffin-embedded blocks were sliced into 2.5-3 µm thickness.

Immunohistochemical staining was done on formalin-fixed, paraffin-embedded tissue sections by an immunoperoxidase method using the Avidin Biotin Complex. Breast carcinoma tissue positively expressing HER2 was used as the positive control and normal breast tissue not expressing HER2 was used as the negative control. An appropriate scoring system, exclusive for gastric tumours, proposed by Hofmann M et al., was the criteria followed and we considered only those tumours expressing 3+positivity as those expressing HER2 [Table/Fig-3] [9]. Their study suggested that this scoring system has a high level of concordance of about 93.5% between IHC and FISH methodologies to detect HER2 positivity in gastric cancer.

Score to report	HER2 protein over expression assessment	Staining pattern
0	Negative	No reactivity or membranous reactivity in <10% of tumour cells.
1+	Negative	Faint or barely perceptive membranous reactivity in >10% of tumour cells, cells are reactive only in part of their membrane.
2+	Equivocal	Weak to moderate complete, basolateral or lateral membranous reactivity in >10% of tumour cells.
3+	Positive	Strong complete, basolateral or lateral membranous reactivity in >10% of tumour cells.

[Table/Fig-3]: HER2 scoring criteria proposed by Hofmann M et al., [9].

STATISTICAL ANALYSIS

All the data was entered in a Data Collection Proforma sheet and were entered into Excel (MS Excel 2010). Statistical analysis was done using SPSS version 19.0 (IBM SPSS, US) software. The expression of HER2 was correlated with variables like age, gender and intestinal and diffuse histological types. Chi-square test was used to compare two groups and p-values <0.05 was considered significant.

RESULTS

Total number of cases taken for our study were 50 with a male preponderance. Age of the cases ranged from 28 to 80 years, with the mean age being 60.76 years. The majority of the cases (42%) were in the age group of 66-80 years, followed by cases from 46 to 65 years (38%) and 30-45 years (20%).

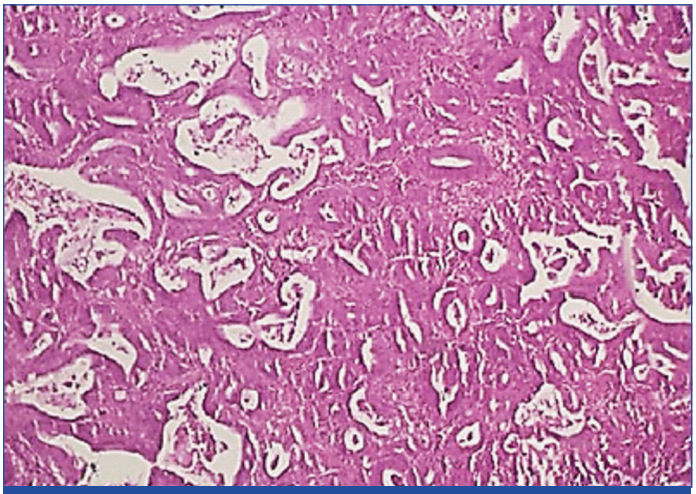
Lauren in the year 1965 classified gastric carcinomas into Intestinal and Diffuse types. In the total of 50 number of specimens, 56% were of intestinal type carcinomas the remaining 44% were that of diffuse type gastric carcinomas. The results of the different clinicopathological variables along with p-values assessed in the study have been presented in the [Table/Fig-4]. The number of cases showing HER2 positivity in Lauren's histological types is also shown. HER2 score was significantly higher in intestinal type gastric carcinomas. Of the total 26 cases expressing the HER2 protein, 81% (21/26) were of intestinal type Vs 19% (5/26) that of diffuse types (p=0.007). Also, no statistical difference was found-between other variables like age and gender (p=0.098 and p=0.985).

Type of tumour	0	1+	2+	3+	Percentage	
By Lauren's type						p=0.007 (Chi -12.199)
Intestinal type	1	3	3	21	75	
Diffuse type	5	5	7	5	25	
By Age						p=0.0980 (Chi -10.714)
30-45 years	0	4	3	3	20	
46- 65 years	4	2	2	11	38	
66- 80 years	2	1	5	13	42	
By Gender						p=0.9850 (Chi-0.153)
Males	4	5	6	16	62	
Females	2	3	4	10	38	

[Table/Fig-4]: HER2 expression and different clinicopathological features of the studied gastric carcinomas.

DISCUSSION

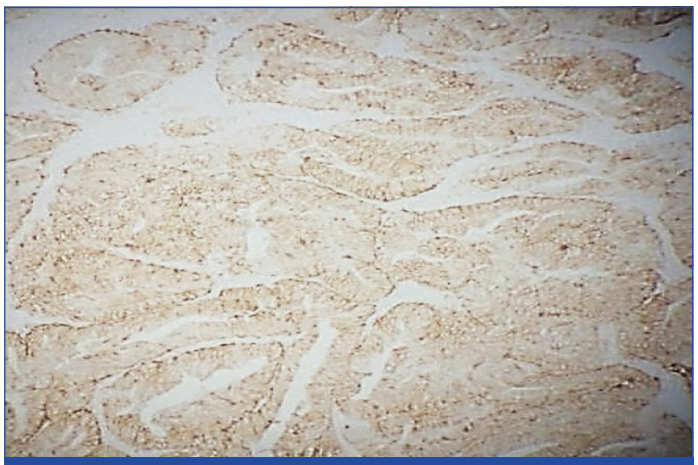
Over the past five decades or more, the mortality associated with gastric cancer has decreased markedly in most areas of the world [10]. There is an increasing incidence of adenocarcinomas of the gastric cardia and gastroesophageal junction, with a marked decline in fundic and distal tumors [10]. Tumours in the gastric cardia have a poorer prognosis with lower 5 year survival [10]. The HER2 gene encodes a 185-kDa transmembrane tyrosine kinase receptor protein, which is a member of the HER-family. HER2 protein is a growth factor of the EGFR family with intrinsic protein tyrosine kinase activity and its increased activity is an assumed mechanism of cell transformation. Activation of EGFR triggers a network of signalling processes that promote tumour cell proliferation, migration, adhesion, angiogenesis and decrease in apoptosis [11]. HER2 overexpression is increasingly recognised as a frequent molecular abnormality in gastric cancers, propelled as in breast cancers by gene amplification and it has been correlated to more aggressive disease and poor outcomes. In this study, we have analysed HER2 expression in gastric carcinomas using IHC among the clinicopathological variables like age and gender, and two histological types as per Lauren's classification, namely Intestinal and diffuse types. In the study done by Fisher SB et al., and Dang HZ et al., the mean ages were 64 and 54 respectively, and the male to female ratios were 1.17:1 and 3.01:1 respectively with an overall male preponderance [12,13]. In our present study the male to female ratio is about 1.63:1, with an overall male preponderance. Henceforth our present study is well correlated to the studies done by other authors. In every region of the globe, gastric cancer has a higher incidence in males than females (ratio of 1.5-2.5:1) [10]. In our present study, we classified gastric carcinomas based on Lauren's type of histological classification, which classifies gastric carcinomas into Intestinal [Table/Fig-5] and Diffuse types. Intestinal type shows well formed tubular formations and diffuse type shows signet ring cell differentiation.



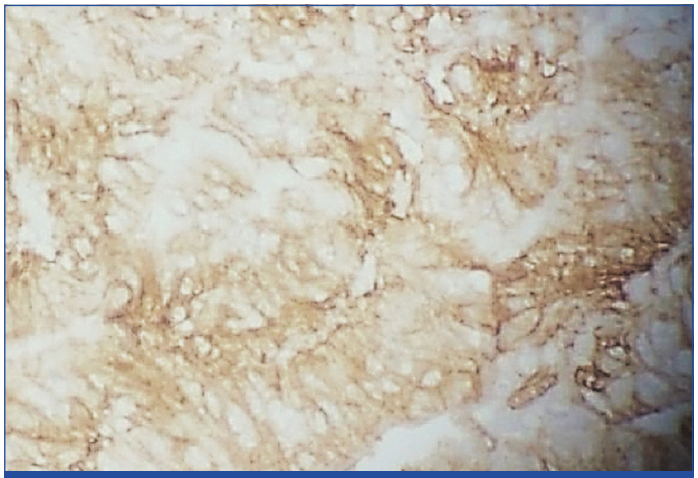
[Table/Fig-5]: Photomicrograph showing intestinal type adenocarcinoma (H&E 10X).

In the studies done by Gravalos C et al., and by Dang HZ et al., the percentage of Intestinal type of gastric carcinomas were 47% and 63%, whereas that of diffuse type were 33% and 37% respectively [11,13]. In our study the intestinal and diffuse types were 56% and 44% respectively. Our study is thus well comparable to other studies regarding the case selection for Lauren's type of gastric carcinomas. Literature shows an extremely variable expression of HER2 in gastric carcinomas ranging from 7% to 43% positivity [14]. In our study 75% of the intestinal type of gastric carcinomas expressed HER2 positivity [Table/Fig-6,7] and the difference in the positivity rate between the histological types-was found to be statistically significant (p=0.007).

In the study done by Tanner M et al., on 131 patients HER2 was overexpressed in intestinal type gastric carcinomas with



[Table/Fig-6]: Showing 3+positivity that of resected specimen (Intestinal type) (IHC 10X).



[Table/Fig-7]: Showing 3+ positivity that of biopsied material (Intestinal type) (IHC 40X).

a statistical significance (p=0.0051) [15]. Presence of HER2 amplification was significantly associated with poor carcinoma specific survival. In the studies done by Fan XS et al., and Salam RA et al., also there was a statistically significant expression of intestinal type gastric carcinomas (p<0.001 and p=0.030) [16,17]. In the studies done by Sekaran A et al., in the year 2012, 44.2% of gastric carcinomas expressed HER2 protein but there was no statistical significance regarding the histological subtypes [18]. The discrepancy in expression of HER2 positivity could be related to the different populations studied. A higher rate of HER2 positivity in our study could be due to increased number of cases from the gastric cardia. The variable data could be attributed to several factors including the usage of different antibodies, varying sample sizes, varying aetiological factors and varying scoring system for interpretation by others. In the studies done by Gravolos C et al., Tanner M et al, and Lordick F et al., the percentage of tumours arising from the gastroesophageal junction expressing the HER2 antigen were 24, 25 and 32 respectively [11,15,19]. In the multicentric international trial the "Trastuzumab for Gastric Cancer 'TOGA [20]- study which was conducted in 130 centres all over the globe including India, they found that about 32% of the tumours arising from the gastroesophageal junction positively expressed the HER2 antigen. Thus, in our study the higher percentage of tumours expressing HER2 could be due to the fact that an increased number of cases were from gastric cardia which lie in a very close proximity to the gastroesophageal junction. Also, in our study there was no statistical significance regarding the clinicopathological parameters of age and gender (p=0.098 and p=0.985). Studies done by Tanner M et al., Salam RA eet al., and Sekaran et al., also showed a statistical insignificance between age and gender in overexpressing HER2 protein [15,17,18].

LIMITATION

One of the limitation of our study was the lack of confirmatory fluorescent in situ hybridization. Moreover, in the future the study with a larger sample size and with a clinical correlation on those on trastuzumab therapy will give a better insight.

CONCLUSION

This study assessed the expression of HER2 in gastric carcinomas and we found a statistically significant expression in intestinal type gastric carcinomas than the diffuse type gastric carcinomas. There was no significant overexpression of HER2 protein regarding the clinicopathological variables like age and gender.

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