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EVALUATION OF EXPRESSION OF HER2/NEU IN GASTRIC CANCER AND ITS SIGNIFICANCE AS A PROGNOSTIC FACTOR IN GASTRIC ADENOCARCINOMA

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ABSTRACT

Context: Gastric cancer is the third leading cause of cancer mortality worldwide. Human epidermal growth factor receptor 2 (HER2) involved in the pathogenesis of several types of cancer, including gastric cancer. Overexpression of HER2 is noted in 10%–22.8% of gastric adenocarcinoma and its identification is of prime importance in targeted therapy. **Aim:** This study aimed to investigate the status of HER-*2/neu* gene in the gastric adenocarcinoma samples and to correlate with various clinico-pathological variables. **Methods and Material:** In this study 50 paraffin embedded tissue blocks from patients with gastric adenocarcinoma [2012–2014] were included. Overexpression of Her2/*neu* was measured by immunohistochemistry. **Statistical analysis:** Data were statistically analyzed by fisher t test and chi- square test using the SPSS statistical software program (Version 19.0) for Microsoft Windows 8. *p* value less than 0.05 considered to be positive. **Results:** Most of gastric carcinomas occurred in male (34 cases), having a mean age of 57 years. A total of eight cases (16%) had expressed a score of 3+ HER2 positivity. HER2 score of 3+ was noted in tumours arise from body and OGJ in six cases, whereas two cases show positivity in pyloric antral tumours. Among histological forms according to WHO classification, 40% (four cases) of signet ring cell carcinomas showed HER2neu expression. **Conclusions:** HER2 overexpression was noticeably associated with signet cell subtype, and tumours arise from body and OGJ.

KEYWORDS: Gastric cancer, HER2, Immunohistochemistry, Prognosis, Therapy.

INTRODUCTION

Gastric carcinoma is the third common cause of cancer related deaths and is responsible for 783,000 cancer deaths in 2018.[1] Gastric Cancers are the second most common cancers in men and third-most common in women in asia.[2] Predominant gastric malignancies (95%) are epithelial in origin.[3] The age related incidence rises sharply and peaks in the age group of 60-80 years. In India, gastric cancers are common in age group of 45 to 55 years in north India and 35 to 55 years in south India. Males are more prone to gastric cancer than females.[4]

Advanced gastric cancers are strongly associated with poor outcome with a median survival of patients with metastasis will be seven to ten months from initial diagnosis. TNM stage is a dependable prognostic tool in gastric carcinoma patients, but patients with similar TNM-stages have been observed to have different clinical outcomes. Even with aggressive chemotherapy, recurrences are common in these patients. Average five year survival rate remains 31%.[5] The present regimens of chemotherapy give unsatisfying results, hence newer therapeutic targets are desired to improve the survival rate. One such example is the drug targeting the human epidermal growth factor receptor2 (HER2) in patients with breast cancer. The HER-2/*neu* gene is located on the long arm of chromosome 17 (17q12-21.32). It encodes p185 oncoprotein which is a receptor tyrosine kinase and it activates a cascade of cellular pathways that contribute to cell growth, proliferation & survival. It has been found to be overexpressed in many types of human malignancies, notably breast, ovarian, gastric, pancreatic, prostatic, colorectal, cancers of the female genital tract and lung cancer. In breast carcinoma, HER2 is a biomarker that has both prognostic and predictive value.[6,7] The survival time of patients with breast carcinoma and positive HER2 disease is significantly shorter than that those with HER2 negative tumour. Although various therapies for gastric carcinoma are available, in recent years molecular target therapy is a new treatment modality for gastric cancer and HER2 has been identified as a potential therapeutic target. Over expression of HER2 receptor in gastric carcinoma using immunohistochemistry is detected in 1986 and has been recognised as an important prognostic factor.[8]

Thus detection of HER2 status is of greater significance in the diagnosis of gastric carcinoma.

Due to genetic heterogeneity of gastric carcinoma, the HER2 over expression and amplification are different when compared to breast carcinoma. The prognostic

significance and prevalence of HER2 in patients with gastric carcinoma is less established than in breast cancer. The prognostic significance of HER2 in gastric carcinoma is still under study. The National Comprehensive Cancer Network (NCCN) guidelines panel recommended that less than 3+ HER2-neu overexpression of by IHC should be additionally examined by FISH or other in situ hybridization methods. This methodology was not widely accepted until 2010. In this study, the expression of HER-2 in gastric cancer is studied by immunohistochemistry. The correlation between HER-2 expression and the clinicopathological parameters of the patients are analysed.

Subjects and Methods

This study was a descriptive study conducted in the Department of Pathology of Tirunelveli government Medical College Hospital, Tirunelveli, Tamilnadu, India during the period from January 2012 to August 2014. Ethical approval for this study (Ethical Committee Numb 299/PATHO/IEC/2012) was provided by the TVMC Research Ethical Committee of Tirunelveli Medical college hospital, tamilnadu, on 14 december 2012. Cases were selected from surgical Pathology division in a retrospective and prospective way. A total cases of 57 gastrectomy specimens were identified during the study period. Among the cases, 52 cases diagnosed as having gastric adenocarcinomas, three cases diagnosed as gastro intestinal stromal tumour, two cases diagnosed as Lymphoma. Among 52 cases, only 50 cases had sufficient remaining tissue in the paraffin block were identified and selected for this study.

INCLUSION CRITERIA

Gastrectomy specimen diagnosed as primary Gastric adenocarcinoma.

EXCLUSION CRITERIA

Specimens diagnosed as non-epithelial tumours, secondary tumours, small cell carcinoma, squamous cell carcinoma were excluded.

METHODOLOGY

We selected 50 cases of gastric adenocarcinoma from patients who had undergone gastrectomy. Clinico-pathological parameters of the patients were then recorded. All 50 cases had their histopathological reports retrieved and the original H&E stained slides were simultaneously reviewed. Tumours were histologically classified as per WHO classification and lauren's classification. The blocks were studied and representative sections were identified for immunohistochemical analysis.

The paraffin-embedded blocks were cut into thin three microns sections, mounted on slides and stained with HER-2neu antibody as per standard protocol as follows: Three µm thick sections obtained from paraffin embedded blocks and sections taken on poly L- lysine coated adhesive slides were deparaffinized in xylene, rehydrated through an ethanol series, and treated in pressure cooker containing citrate buffer (pH6.0) for antigen retrieval, cooled for 20 minutes and washed with a buffer solution. Peroxidase was applied for five minutes and washed with a buffer solution for ten minutes to reduce the nonspecific staining due to endogenous peroxidase activity.

Then protein block was applied and incubated for five minutes to block the nonspecific background staining. Rabbit anti human polyclonal Her2 antibody (thermo fisher scientific) is then added over the tissue and incubated for 30 minutes. Followed by the primary horse radish peroxidase polymer amplifier is added for 20 minutes to enhance the process of primary antibody which is then washed in TRIS wash buffer. Secondary antibody is added and incubated for 20 minutes and then washed with TRIS wash buffer. The bound antibody was visualized using a diaminobenzidine (DAB) chromogen and incubate for five minutes. Then counterstaining was done with hematoxylin for 30 seconds and washed in tap water. Mounting is done by DPX mountant. Section stained by omission of the primary antibody was used as a negative control. Over-expression of HER-2 protein paraffin-embedded invasive breast carcinoma tissue slides was used as a positive control.

A strong brown staining was found in cell membrane of malignant cells using this staining method. Immunohistochemistry stained sections was examined and evaluated manually by two experienced pathologists, according to the scoring system by Hofmann *et al.*[9] We used membrane stain graduation scale for assess the IHC. It is graded 0, 1+, 2+, 3+ according to the panel scoring (Table 1).[9] Only membranous pattern of expression was considered for evaluation. Score 0 for no reactivity or membranous reactivity in less than 10% of tumour cells. Score 1 for faint /barely perceptible membranous reactivity in >10% of tumour cells. Score 2 for weak to moderate membranous reactivity in >10% of tumour cells. Score 3 for moderate to strong membranous reactivity in >10% of tumour cells. All cases with score 3+ were considered positive. Cases scored as 2+ were considered equivocal for HER2 expression, whereas 0 and 1+ cases were considered as negative.

Statistical analysis

The correlations between HER2 status and patient clinico pathological data was evaluated by using fisher t test and chi- square test. p value less than 0.05 considered to be positive. Data has been analysed by using the SPSS statistical software program (Version 19.0) for Microsoft Windows 8.

RESULTS

A total of 50 patients were included in this study group who fulfilled the inclusion criteria. A partial gastrectomy

was done in 45 cases and total gastrectomy was done in five cases. All cases histopathologically diagnosed to be gastric adenocarcinoma. Clinicopathological characteristics are depicted in Table 2.

The age range of the study group was 37–87 years with mean age 57 years. Majority of patients were male, sex ratio (male: female) being 2:1. All specimens were of complete or partial gastrectomy. Tumours were located at the cardiac end (one case, 2%), body (15cases, 30%), OGJ (two cases, 4%), and pyloric antrum (32 cases, 64%). Routine histopathological examination with H and E staining revealed gastric adenocarcinoma and were grouped according to Lauren classification. Intestinal type of adenocarcinoma [Figure 1] comprised 32/50 cases (64%), whereas the diffuse type of adenocarcinoma [Figure 2] was diagnosed in 18/50 cases (36%). Regarding grading of tumours, well and moderately differentiated adenocarcinoma was noted in 26/50 patients (52%) and poorly differentiated adenocarcinoma accounted for 24/50 cases (48%).

Out of 50 cases which were studied for the HER2/neu reactivity, four cases showed 3+ (8%), four cases showed 2+ (8%), 16 cases showed 1+(32%), and 26 cases (52%) did not show reactivity (negative) as per the scoring system devised by Hofmann scoring system. [Table 3].

Immunohistochemistry for HER2/neu showed a score of 3+ positivity [Figure 3 & Figure 4] in eight cases (16%), which originated from the body and OGJ (six cases), pyloric antrum (two cases). [Table 4].

Among histological forms, according to WHO classification 15% of tubular carcinomas, 16.6% of papillary carcinomas, 40% of signet ring cell carcinomas showed HER2neu expression. Mucinous carcinoma and diffuse type carcinoma showed negative for HER2neu expression. [Table 5].

22 patients had a primary tumour size of less than that of median tumour size five cm, of which three cases(13.6%) shows positivity for HER2 expression. 28 patients had a primary tumour size of more than five cm, of which five cases(17.9%) shows positivity for HER2 expression.

27 cases had more than 0.5 LN ratio, of which six cases (22.22%) showed positivity for HER2 protein.

34 cases showed lymphatic invasion. Out of 34 cases, HER2 expression was noted in seven cases (20.6%). And 16 cases without lymphatic invasion also shows 6.25% (1 case) positivity for HER2 expression.

According to seventh edition of UICC guidelines, pTNM staging of 50 cases were done. Stage evaluation revealed that six cases falls under stage I, 24 cases in stage II & 20 cases in stage III. Five out of 20 cases in stage III(25%) had shown positive for HER 2 expression. Three out of 24 cases(12.5%) in stage II were defined positivity for HER2 expression, while none of the stage I cases revealed HER2 expression.

The medical records of each case was reviewed and medical, demographic and pathological data of the patients was collected. Patient's age and sex were included in demographic data. Tumour location and TNM stage and treatment history were included in Clinical and pathological data. Clinico-pathological variables of gastric cancer cases with HER-2 expression demonstrated in Table 6 and Table 7.

In this study HER 2/neu expression was correlated with Histological type- signet ring cell carcinoma (p value < 0.05). HER 2/neu expression was also correlated with location of the tumour. OGJ and corpus tumours are associated with HER2 neu expression compared with antral- pyloric tumour (p value < 0.05). And there were no differences between the groups in terms of age, gender, type of gastrectomy, tumour size, nodal status, stage of disease, Perineural invasion, serosal invasion and differentiation of tumour.

Table 1: Consensus recommendations on HER-2 scoring for gastric cancer (Hofmann *et al*).

DEFINITION	SCORE
No reactivity or membranous reaction in <10% of cells	0 / negative
Faint/barely perceptible membranous reactivity in >10% of cells. Cells are reactive only in part of their membrane	1+ /negative
Weak to moderate complete or basolateral membranous reactivity in >10% of cells	2+ /equivocal
Moderate to strong complete or basolateral membranous reactivity in >10% of cells	3+ /positive

Table 2: Clinico-pathological characterization of gastrectomy cases.

VARIABLE/ PARAMETER	*n* (%)
Age(years) Median-57 years	
≥57	28 (56)
<57	22 (44)
Gender(*n*)	
Men	34 (68)
Women	16 (32)
Lauren phenotype, (*n*)	
Intestinal	32 (64)
Diffuse	18 (36)
Localisation, (*n*)	
Body	15 (30)
Pyloric antrum	32 (64)
OGJ	2 (4)
Cardia	1 (2)
TNM stage(7^{TH} edition) , (*n*)	
Stage I	6 (12)
Stage II	24 (48)
Stage III	20 (40)
Lymph node ratio	
Median	0.5
Lymphatic invasion, (*n*)	
pL0	16 (32)
pL1	34 (68)
Total Cases (n)	50

Table 3: Number HER2 Reactivity Cases In This Study.

HER2/*neu* score	Number of cases (*n*)	Percentage (%)
0	26	52
1+	16	32
2+	4	8
3+	4	8

Table 4: HER2 Expression According to Location of Tumour.

Tumour Location	Total cases(*n*)	HER2 negative(*n*)	HER2 positive(*n*)
Body & OGJ	17	11	6
Pyloric antrum	33	31	2
TOTAL	50	42	8

Table 5: Signet Cell Carcinoma (SRC) & HER2 Expression.

Histological type	Total cases(*n*)	HER2 negative(*n*)	HER2 positive(*n*)
SRC	10	6	4
Non SRC	40	36	4
Total	50	42	8

Table 6: Clinico-pathological variables of gastric cancer patients stratified by HER-2 status.

Parameter	*HER2 negative*	*HER2 positive*	*P-value*
Age(years)			0.439
<57	20(40)	2(4)	
≥57	22(44)	6(12)	
Gender			0.699
Men	29(58)	5(10)	
Women	13(26)	3(6)	
Localisation Body Pyloric antrum OGJ	 10(20) 31(62) 1(2)	 5(10) 2(4) 1(2)	0.027

Tumour size			1.000
<5cm	9(38)	3(6)	
≥5cm	23(46)	5(10)	
Differentiation			1.000
Yes	22(44)	4(8)	
No	20(40)	4(8)	
Lauren's classification			0.4357
Intestinal	28(56)	4(8)	
Diffuse	14(28)	4(8)	
Lymph node ratio			0.2609
<0.5	21(42)	2(4)	
≥0.5	21(42)	6(12)	
Perineural invasion			0.1968
No	32(64)	4(8)	
Yes	10(20)	4(8)	
Angio lymphatic invasion			0.4092
No	15(30)	1(2)	
Yes	27(52)	7(14)	

Table 7: HER-2 Positivity according to TNM Stage (According to 7th edition).

TNM Stage	*HER2 negative*	*HER2 positive*	*P-value*
Stage I	6(12)	0	
Stage II	21(42)	3(6)	0.277
Stage III	15(30)	5(10)	

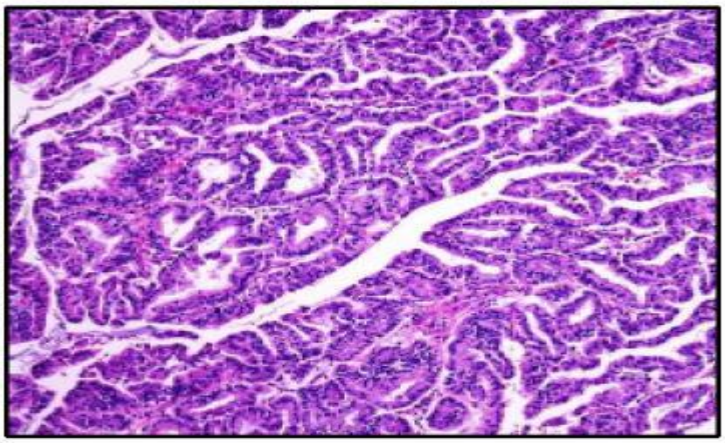

Figure 1: Gastric Adenocarcinoma- Intestinal type (100X).

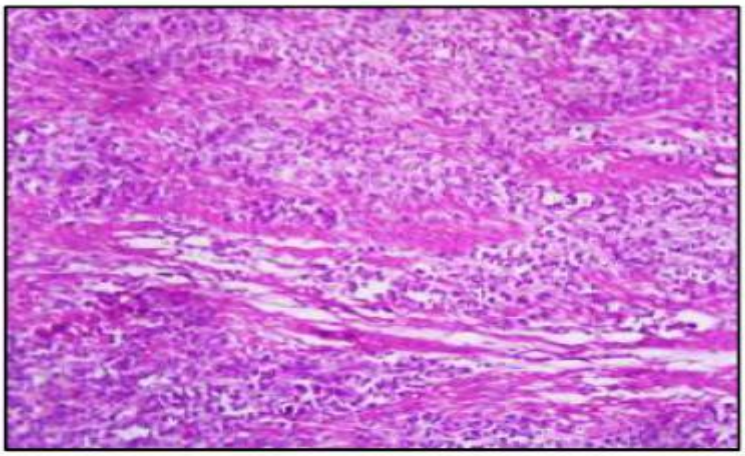

Figure 2: Gastric Adenocarcinoma—Diffuse Type (100X).

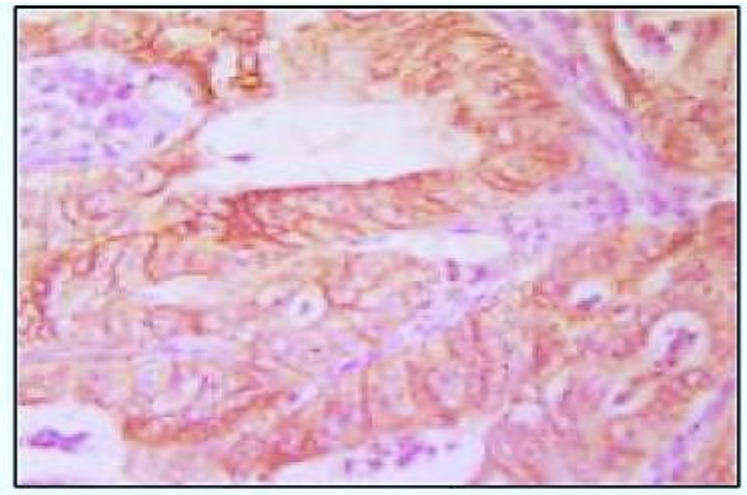

Figure 3: Photomicrograph showing expression of HER2/neu in intestinal type of adenocarcinoma.

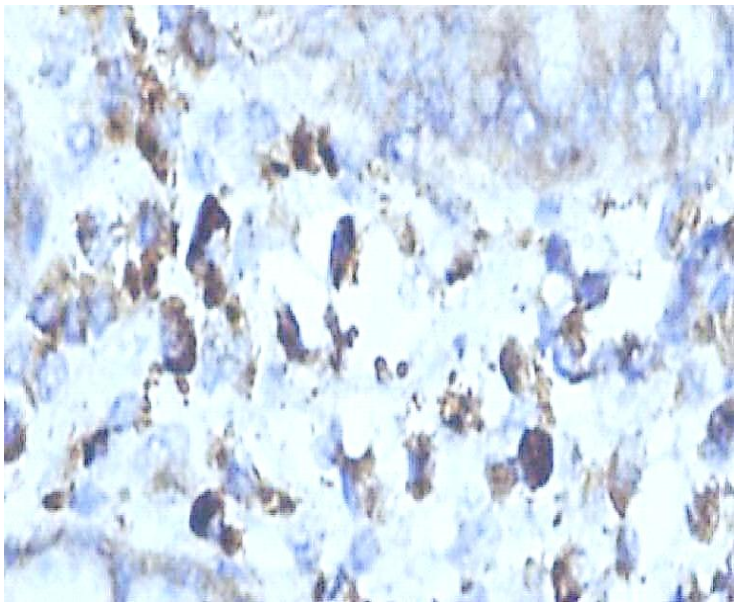

Figure 4: Photomicrograph showing expression of HER2/neu in signet ring cell adenocarcinoma.

DISCUSSION

Worldwide prevalence rates of over expression of HER2 in gastric cancer varies from 8.2% to 62.5% in different reports.[10] The survival time of patients with breast carcinoma and positive HER2 disease is significantly shorter than that those with HER2 negative tumour.[11,12] Thus detection of HER2 status is of greater significance in diagnosis and prognosis of gastric carcinoma.

The pressing clinical question is whether or not HER2 expression confers prognostic information. Some studies revealed HER2 appear to be a valuable prognostic factor. In literature, few studies have shown a strong association between HER2 over expression and worse prognosis.[13,14]

In this study, over expression of HER2/*neu* protein observed in 8 cases(16%) of gastric carcinoma specimens as determined by IHC. we have found a statistically significant association between HER2/neu positivity with tumour location and signet ring cell carcinoma according to WHO classification.

It shows correlation with higher rate of HER2 positivity in OGJ & corpus cancer than antral-pylorus cancer (35.29% Vs 6.06% respectively) with a p value <0.05, and it is consistent with results of other studies.

M.Tanner et al 2005.[15] found that positive rates of HER2 ranges from 24% to 12% for tumours located at the gastro oesophageal junction or in other areas of the stomach, respectively. The 2009 ToGA (Trastuzumab for Gastric Cancer) trial also revealed in the same way and showed a positivity which ranges between 32% and18%, respectively.[16] According to ToGA trail (2009), the countries with the increased ratio of OGJ: gastric cancer were found to have above average HER2 positivity rates, irrespective of sample size.

In contrast, Marx Andreas H., et al (2009) found that there was no statistically significant difference between

HER2 positivity and tumour site.[17] And De Carli *et al*. found 85.7% HER2/neu positivity in pyloric antrum[1][8], Lakshmi *et al*. found 51.2% positivity in pyloric antrum.[19]

In this study we have also found statistically significant association between signet ring cell carcinoma and HER2/neu expression (40%), with p value of <0.05. Conflicting evidence regarding HER2 and signet ring cell features exists.

Cangiano J et al 2008[20] study found that tumours showing signet ring cell features uniformly overexpressed HER2 as measured by IHC, although the sample size was not reported and determination of HER2 protein status relied on the breast cancer scoring system for IHC without molecular analysis (FISH). Future studies should make an effort to quantify the association between signet ring cell features and HER2 status.

In contrast, Grabsch H et al 2010[10] reported that there is no significant correlation between signet cell features and HER2 expression.

In this study there was no significant correlation between HER2neu expression and the other clinicopathological prognostic factors , such as age, gender, similar to the studies done by M.Tanner et al 2005[15] and Ananiev Julian, et al 2011.[21]

In this study, no relationship was found between HER2neu expression and primary tumour size, TNM staging, nodal status, lymph node ratio, Lauren's classification and it is consistent with of other studies.

Ghaderi, Abbas, et al. Reported that except tumour stage, no other correlation was observed between the HER2 neu overexpression and the studied variabels.[22]

The other study by Halon et al., (2012) demonstrated that only TNM stage and patients' age were crucial negative prognostic factors.[23]

In contrast, Wang Yuan-Yu, et al[24] observed Positive expression of HER2neu correlated with age, size of tumour, location of tumour, depth of invasion, vessel invasion, lymph node, and distant metastasis and TNM stage.

Yu GZ et al 2009 also observed significant differences in Her2 expression between the primary tumours and the lymph node metastases[25] ($P < 0.01$).

Kang et al[26] reported that HER-2 overexpression correlated with the histological type as in Lauren's classification with 34% intestinal type; 6% diffuse type.

Due to significant differences in the historical studies, the role of HER2 as a prognostic marker in gastric carcinoma has been controversial. More recent studies found that HER2 is an important poor prognostic factor in gastric cancer patients.[9,27,28,29]

In this study we found that the Histological type of signet ring cell carcinoma, act as a as an independent prognostic factor and tumour located in Oesophageal gastric junction & corpus location of the tumour are the candidate parameters for prognostication.

Our study has limitations because of small sample size and non-use of FISH technique to confirm the HER2 expression in 2+ equivocal cases. This techniques added more evidence for targeted therapy.

This study concludes that HER2 status has a significant role in prognosis of gastric adenocarcinoma and may be considered as an independent prognostic factor in gastric carcinoma patients. So further study is needed to explain the role of HER2 on development and prognosis of gastric cancer.

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