

A Study of HER-2/neu Expression in Gastric Carcinoma in a Tertiary Care Hospital in North Telangana

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ABSTRACT

Background: Gastric cancer is the fifth most common cancer and the 3rd most common cause of cancer related mortality worldwide. Gastric carcinoma contributes to 10% of cancer deaths worldwide with a case fatality ratio of 70%. Despite recent advances in cancer treatment, the overall 5-year survival for patients with newly diagnosed gastric cancer is 28%. **Objective:** To study the HER-2/neu expression in gastric carcinoma patients in a tertiary care hospital.

Materials and Methods: The present study is a retrospective and prospective study done to assess HER2/neu protein over expression in gastric carcinomas. Excision specimens of patients with gastric carcinoma who underwent surgery were obtained from cancer hospital and research institute were included in the time bound study.

Results: 50 Gastrectomies were included in this study. The histological features correlated with HER2/neu overexpression. The youngest patient was 38 years old and the oldest was 74 years. The mean age of the patients was 57.68 years. All the cases were adenocarcinomas. Most of the tumors, i.e., 35 cases were of intestinal type of adenocarcinomas, whereas 15 cases were of diffuse type.

Conclusion: Our study found HER2/neu over expression of 10.0% in gastric cancers similar to most studies in India and the rest of the world. Additional research is required to explore role of HER2/neu as an independent prognostic factor and to confirm any existing geographic variation. It is important that pathologists and diagnostic laboratories learn and use the modified criteria for evaluating HER2/neu status in gastric /GEJ carcinomas. Large number of new specimens will need to be examined for HER2/neu status using both FISH and IHC in the days to come as trastuzumab has now been validated by the Food and Drug Administration (FDA) for management of metastatic gastric adenocarcinomas.

Keywords: Gastric carcinoma, HER2neu, adenocarcinoma.

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INTRODUCTION

Gastric carcinoma is the 5th most common cancer and third leading cause of cancer related death in the world. In India it ranks as the 5th most common malignancy in males and 7th most common in females. Gastric carcinoma is the second leading cause of death in both sexes. Scientists in their persistent effort to probe into the cause of cancer have made significant inroads into the phenomena and are on the road to success to decipher its cause and find a cure.^[1]

Gastric carcinoma contributes to 10% of cancer deaths worldwide with a case fatality ratio of 70%.^[2] Despite recent advances in cancer treatment, the overall 5-year survival for patients with newly diagnosed gastric cancer is 28%.^[3]

Continuous efforts are put to identify certain specific biological markers that could help in diagnosing the disease early and also help in improving the targeted therapy. Markers like HER2, E-cadherin, EGFR etc. are currently used to evaluate prognosis of disease. HER2 is a proto-oncogene, located in chromosome 17q21. It encodes a transmembrane protein having tyrosine kinase activity. It is also known as ERBB2 and CerbB2. It regulates signal transduction pathway causing cell growth and differentiation. It is a member of epidermal growth factor receptor family. Accurate evaluation of expression of HER2 protein can be a help to identify eligible candidates for new targeted therapy.

Human epidermal growth factor receptor 2 (HER2) overexpression is increasingly recognized as a frequent molecular abnormality in gastric and gastroesophageal cancer. With the recent introduction of HER2 molecular targeted therapy for patients with advanced gastric cancer, determination of HER2 status is crucial in order to select patients who may benefit from this treatment.

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase and a member of the epidermal growth factor receptor family. HER2 is a proto-oncogene encoded by ERBB2 on chromosome 17. HER2 is found in many tissues, including breast, the gastrointestinal tract, kidney, and liver.^[1]

In general, it suppresses apoptosis and promotes cellular proliferation, thus facilitating uncontrolled cell growth, leading to tumorigenesis. HER2 acts as an oncogene in a wide range of cancers. It is now well accepted that HER2 status is of supreme importance in breast cancers, with positivity for HER2 indicating a poor prognosis. HER2 studies have been carried out extensively in breast cancer but not in gastric cancer. With increasing understanding of the molecular biology of HER2 and the availability of genomics and proteomics analyses, it has now been recognized that HER2 is implicated in other severe forms of cancer, notably gastric cancer. Unlike the consensus in breast cancer, results of different studies in gastric cancer have yielded inconsistent findings regarding prognosis, some studies have shown a worse prognosis whereas others found no association.

Objective: To study the HER-2/neu expression in gastric carcinoma patients in a tertiary care hospital.

MATERIALS & METHODS

Study Design: Hospital based Prospective and Retrospective observational study.

Study area: Dept. of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar.

Study Period: July 2016 to August 2017.

Study population: All excision specimens with microscopic evidence of gastric adeno carcinoma and sent for pathological analysis.

Sample size: Study consisted a total of 50 patients.

Sampling method: Simple Random sampling method.

Inclusion Criteria: Patients who underwent surgery for Gastric carcinoma.

Exclusion Criteria: Other carcinomas except for gastric cancers.

Ethical consideration: Institutional Ethical committee permission was taken prior to the commencement of the study.

Study tools and Data collection procedure: All biopsies and non-neoplastic lesions were excluded. Fifty cases were collected during the study period satisfying the inclusion and exclusion criteria. Clinical data, including age,

sex, symptoms and metastatic sites were collected from the case files and medical records. Routine Hematoxylin and Eosin staining was performed initially which includes steps like. Dewaxing, Dehydration, Hematoxylin, Differentiation, Bluing, Eosin, Dehydration, Clearing and Cover-slipping.

Positive control used was breast carcinoma that scored HER2/neu 3+. HER2/neu immunoreactivity was evaluated according to the previously defined scoring system proposed by Hofmann and coworkers and Ruschoff and coworkers. All the cases with 2+ and 3+ scores were considered HER2/neu positive.

Statistical Analysis:

The data was collected, compiled and compared statistically by frequency distribution and percentage proportion. Quantitative data variables were expressed by using Descriptive statistics (Mean \pm SD). Qualitative data variables were expressed by using frequency and Percentage (%). Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Chi-square test has been used to find the significance of study parameters on categorical scale between two or more groups. P values of <0.05 were considered statistically significant. Data analysis was performed by using SPSS Version 20.

RESULTS

50 Gastrectomies were included in this study. The histological features correlated with HER2/neu over expression.

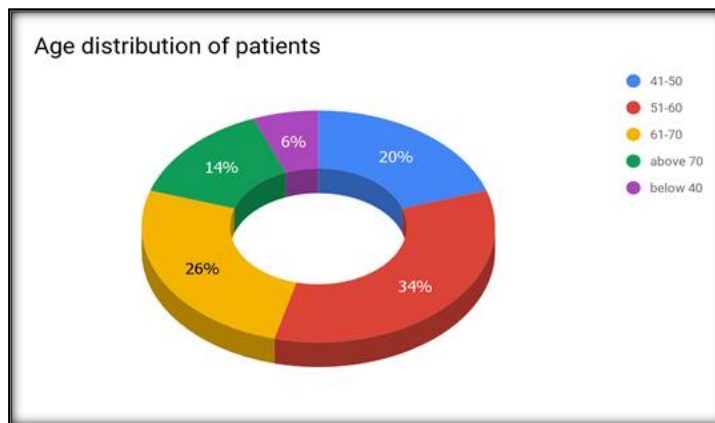


Figure 1: Age distribution of patients

The patients were divided into five groups: 1) those below 40 years. 2) those between 41-50 years, 3) between 51-60 years, 4) between 61-70 years, 5) those above 70 years. Out of 50 cases studied maximum of 34% (17/50) cases were between 51 to 60 years while only 6% were below 40 years. The youngest patient was 38 years old and the oldest was 74 years. The mean age of the patients was 57.68 years. [Standard Deviation (SD) = 12.12].

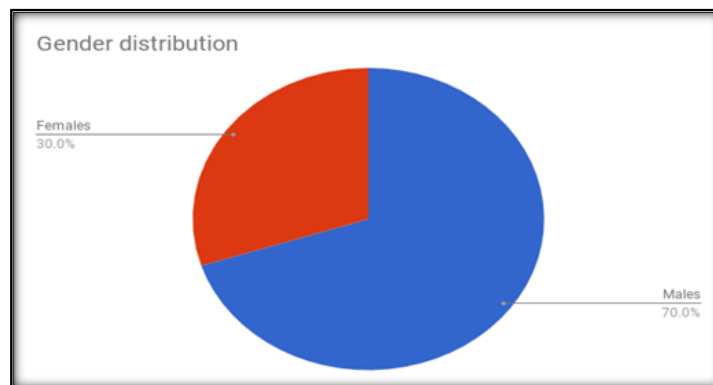


Figure 2: Gender distribution of patients

Type of specimen:

Most (80.0%; 40 /50) of the specimens were distal gastrectomies and 10 (20.0% were total gastrectomies.

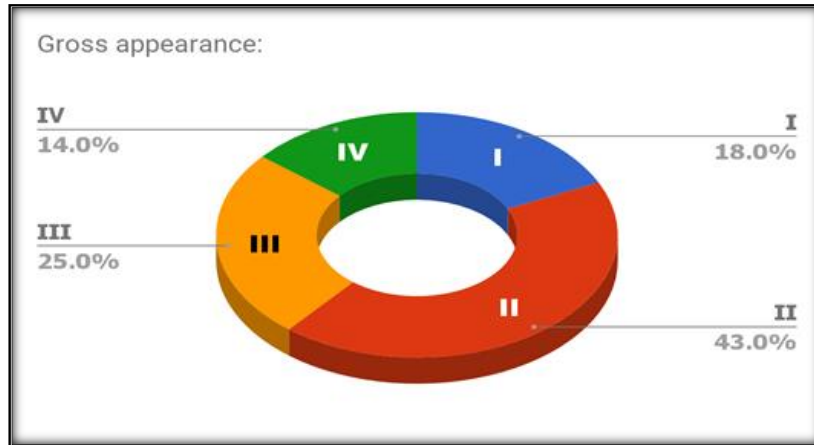


Figure 3: Frequency of tumors according to Bormann's classification

Grossly, of the 50 cases in the study, 18% (9/50), 43 % (20/50), 25% (14/50) and 14% (7/50) belong to Bormann classification type I,II,III,IV respectively.

Size

The tumor size ranged from as small as 0.5cm to as large as 9.5 cm. the average size of the tumor was 4.28cm. (SD=2.09).

Site

Most of the tumors (90% were seen in the antropyloric area. Seven (10%) cases were seen in the body of the stomach.

Lauren's classification

All the cases were adenocarcinomas. Most of the tumors, i.e., 35 cases were of intestinal type of adenocarcinomas, whereas 15 cases were of diffuse type.

Table 1: Grading of intestinal type adenocarcinomas

Grade	Frequency	Percentage (%)
1	12	35
2	18	50
3	5	15
Total	35	100

Out of 50 cases, 23% (11/50), 33 % (17/50) and 44 % (22/50) cases belonged to Grade 1, 2 and 3 respectively (including diffuse type, Grade 3). Of the 35 intestinal type of adenocarcinomas included in our study, 50.0% were moderately differentiated (Grade 2).

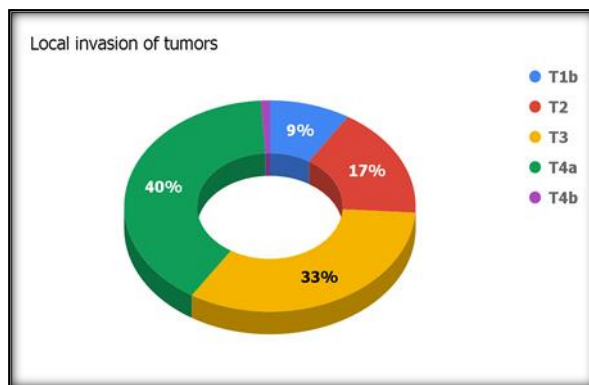


Figure 4: Local invasion of tumors

Most of the tumors (40%, 20/50) were in T4a stage whereas 9% (4/50) of the cases were in the early stage (T1b).

Lymph node status

Sixteen cases (33%) showed a lymph node status of N1, followed by N0 (13 cases, 26%), N3 (11 cases, 22%) and N2 (10 cases, 19%).

Lymph-vascular invasion

Lymph-vascular invasion was present in 15 % (8/50) of cases and absent in 85% (42/50) cases.

Metastasis

2 cases presented with metastatic lesions, involving the liver.

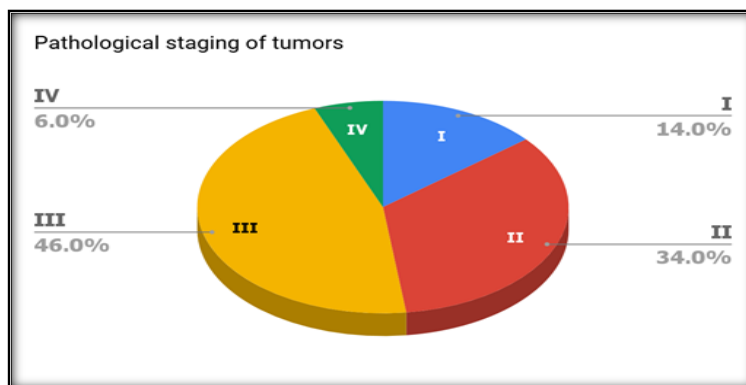


Figure 5: pathological staging of tumors

Of all the cases, 14% (7/50), 34 % (17/50), 46% (23/50), and 6% (3/50), were in pathological TNM stage I, II, III, IV, respectively.

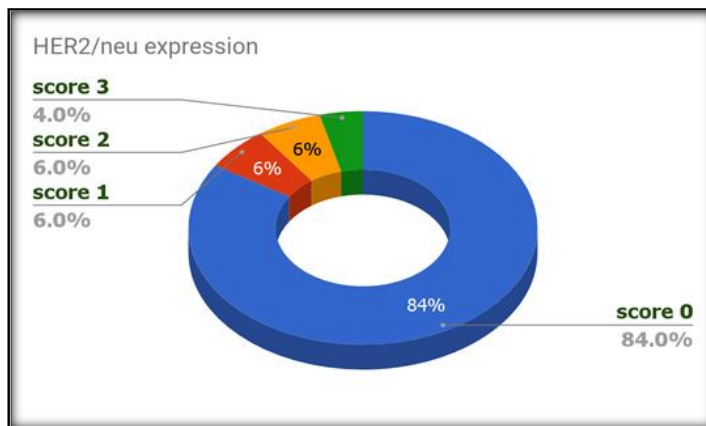


Figure 6: HER2/neu expression in tumors

Table 2: correlation Age with HER2/ neu

Age (in years).		HER2/neu				Total
		0	1+	2+	3+	
40 and below	2	0	0	0	2	
	100.0%	0%	0%	0%	100.0%	
	4.7%	0%	0%	0%	4.7%	
41-50	2	0	0	0	2	
	100.0%	0%	0%	0%	100.0%	
	4.7%	0%	0%	0%	4.7%	
51-60	16	1	1	1	19	
	84.2%	5.2%	5.2%	5.2%	100.0%	
	38.0%	33.3%	33.3%	50.0%	38%	
61-70	12	1	1	0	14	
	85.7%	7.1%	7.1%	0%	100.0%	
	28.5%	33.3%	33.3%	0%	28%	
Above 70	10	1	1	1	13	
	76.9%	7.7%	7.7%	7.7%	100.0%	
	23.8%	33.3%	33.3%	50.0%	26%	
Total	42	3	3	2	50	
	84%	6.0%	6.0%	4.0%	100.0%	
	100.0%	100.0%	100.0%	100.0%	100.0%	

P= 0.972 NS

HER2/neu expression amongst various age groups is shown in table 5. Forty five cases (89%) showed HER-2/neu negativity, and there was no correlation with any age group . In one out of 19 cases (5.2%) in 51-60 years age group and in one case out of 13 cases (7.6 %) in age group above 70 showed score 3 strong positivity for HER2/neu expression in each group.. Whereas one case in each age group from 51-60, 61-70 and above 70 showed score 2 positive HER2/neu expressions.

Table 3: Gender with HER2/neu correlation

Gender		HER2/neu				Total
		0	1+	2+	3+	
Female	14	1	0	0	15	
	93.3%	6.6%	0%	0%	100.0%	
	33.3%	33.3%	0%	0%	30.0%	
Male	28	2	3	2	35	
	80.0%	5.7%	8.5%	5.7%	100.0%	
	66.6%	66.6%	100.0%	100.0%	70.0%	
Total	42	3	3	2	50	
	84.0%	6.0%	6.0%	4.0%	100.0%	
	100.0%	100.0%	100.0%	100.0%	100.0%	

P=0.333NS

HER2/neu over expression of 3+ was seen in two male cases (5.7%) followed by score 2+ in three males (8.5%), score 1+ in one female case (6.6%) and two males (5.7%). Fourteen female cases [93.3%, (14/15)] and twenty-eight male cases (80.0%) were negative with score 0 for HER2/neu expression.

Table 4: Lauren's Classification with HER2/neu correlation.

Laurens Classification		HER2/neu				Total
		0	1+	2+	3+	
	Diffuse	14	0	1	0	15
		93.3%	0%	8.3%	0%	100.0%
		33.3%	0%	50.0%	0%	30.0%
	Intestinal	28	3	2	2	35
		80.0%	8.5%	5.7%	5.7%	100.0%
		66.6%	100.0%	66.6%	100.0%	70.0%
total		42	3	3	2	50
		84.0%	6.0%	6.0%	4.0%	100.0%
		100.0%	100.0%	100.0%	100.0%	100.0%

P= 0.083 NS

All the cases showing strong HER2/neu expression were of intestinal type (9.1%, 2/35).

Two out of 35 cases (5.7%) intestinal type tumors and 1/15 cases (8.3%) of the diffuse type showed weak positivity of score 2+.

Table 5: Pathological stage with HER2/neu correlation

		HER2/neu				Total
		0	1+	2+	3+	
Pathological stage	I	5	0	0	0	5.0%
		100.0%	0%	0%	0%	100.0%
		11.9%	0%	0%	0%	10.0%
	II	16	0	1	0	17
		94.1%	0%	5.8%	0%	100.0%
		38.0%	0%	33.3%	0%	34.0%
	III	20	3	2	2	27
		74.0%	11.1%	7.4%	7.4%	100.0%
		47.6%	100.0%	66.6%	66.6%	54.0%
	IV	1	0	0	0	1
		100.0%	0%	0%	0%	100.0%
		2.3%	0%	0%	0%	2.0%
Total		42	3	3	2	50
		84.0%	6.0%	6.0%	4.0%	100.0%
		100.0%	100.0%	100.0%	100.0%	100.0%

P= 0.474 NS

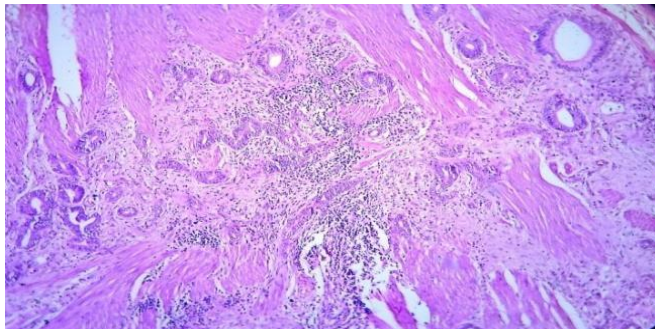
Of the cases which showed strong HER2/neu expression, 100.0% (2/2) belonged to Stage III. One case in Stage II and two cases in Stage III showed weak positivity.

Table 6: Tumor grade with HER2/neu correlation

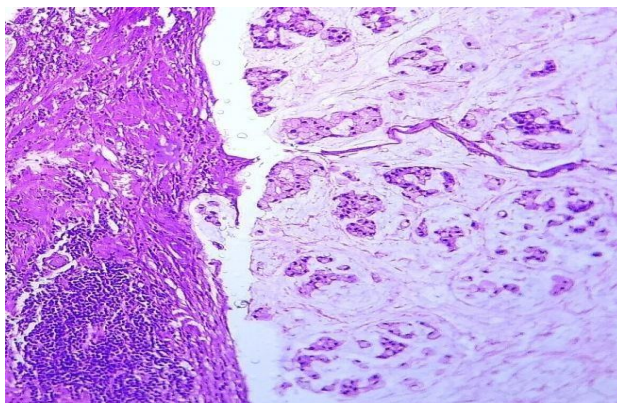
		HER2/neu				Total
		0	1+	2+	3+	
Grade	1	3 50.0% 7.1%	2 33.3% 66.6%	1 16.6% 33.3%	0 0% 0%	6 100.0% 12.0%
	2	14 87.5% 33.3%	0 0% 0%	1 16.2% 33.3%	1 6.2% 50.0%	16 100.0% 32.0%
	3	25 89.0% 59.5%	1 3.5% 16.6%	1 3.5% 33.3%	1 3.5% 50.0%	28 100.0% 56.0%
Total		42 84.0% 100.0%	3 6.0% 100.0%	3 6.0% 100.0%	2 4.0% 100.0%	50 100.0% 100.0%

P= 0.565 NS

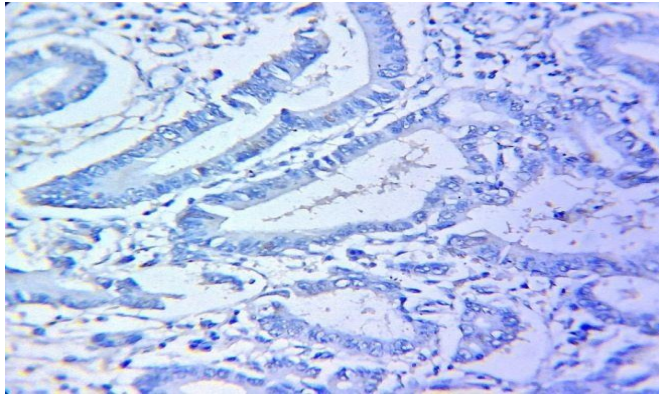
In moderately differentiated and poorly differentiated tumors, score 3+ positivity was seen one case for each grade (1/16, 6.2%) and (1/28, 3.5%) respectively, compared to well differentiated tumors which showed (1/6, 16.6%), score 2 + positivity. so the well and moderately differentiated have shown higher percentage of HER2/neu positivity. This result is observed in our study which correlated with many other studies.



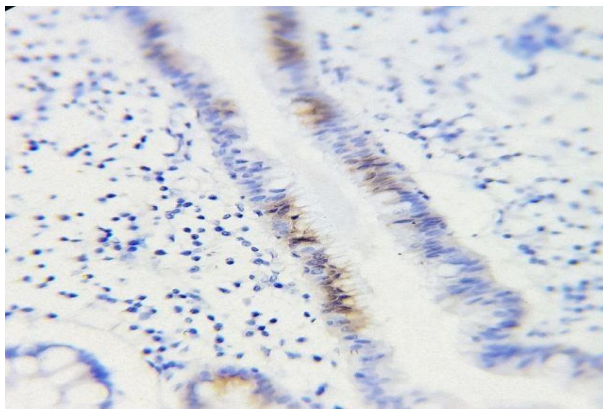
Picture 1: Section showing Intestinal type adenocarcinoma showing neoplastic glands extending up to muscularis propria. (H&E 10x)



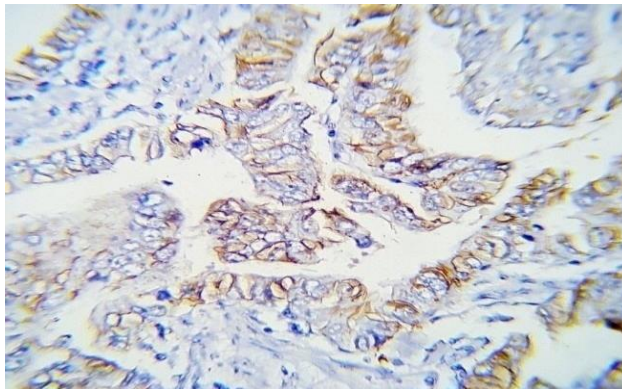
Picture 2: Section showing Mucinous adenocarcinoma with adjacent stroma shows desmoplastic reaction. (H&E, 40x)



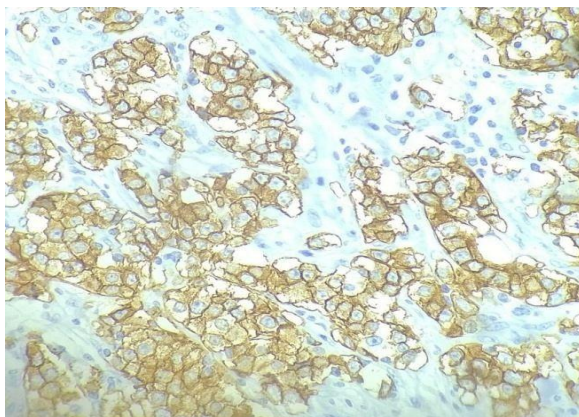
Picture 3: Section showing HER2/ neu score 0 (IHC 40x).



Picture 4 : Section showing HER 2/ neu score 1+ (IHC 40x)



Picture 5: Section showing HER 2/neu score 2+ (IHC 40x)



Picture 6: Section showing HER2/neu score 3+ (IHC 40x)

DISCUSSION

Age of patients in our study ranged from 38-74 years. Most of the patients aged between 51-60 years. Mean age was 57.68 years. Males accounted for 70.0% and females 30.0%. This male female sex ratio of 2:1 correlates with other studies. The age and gender distribution was similar to other studies conducted in various parts of India. Most of the tumors were located in antro-pyloric area of stomach (91.8%) and the rest were in the body (8.2%). The frequency of antral tumor location is slightly higher than the previous studies⁴. The tumor size ranged from 1.5cm to 9.5 cm. The average size of tumor was 4.28 cm (SD=2.09), this result was similar to study by Rakshani and coworkers.^[4]

Majority of tumors (70.0%) was of intestinal type and the rest were of diffuse type (30.0%). Rest of the studies showed a similar frequency as the present study. Poorly differentiated tumors were the predominant (56.0%) type followed by moderately differentiated (32.0%) and well differentiated type (12.0%). This distribution was similar to the results of many studies. However, Rajagopal and coworkers reported a predominance of the moderately difference type.^[3]

In our study, most patients were of pathological stage T4 (56.0%) followed by T3 (28.0%) T2 (12.0%) and T1(4.0%). Regarding the pathologic N stage, 40.0% showed a lymph node status of N1, followed by NO (36.0%) and N2 (16.0%) and N3 (8.0%). The distribution of these pathological variables is slightly different in other studies.^[3,4]

HER2/neu, for prognostication and as a marker for targeted therapy for breast cancers is an established fact. It has also been of interest to many researchers for cancers of the gastrointestinal tract (GIT) since few years. Some studies have reported high frequency of HER2/neu protein overexpression in the tumors of the upper GIT.^[5,6]

The phenotypic and genotypic distinction of cancer is increasingly employed to modify treatment strategies. The introduction of trastuzumab for HER2/neu protein metastatic gastric cancers in the first – line management has added gastric carcinoma to an increasing list of cancers to which molecular targeted therapy can be administered.^[7,8] The evaluation of gastric adenocarcinoma for HER2/neu overexpression with a single immunohistochemical-staining assay was the primary goal of this study. In our study HER2/neu overexpression of gastric adenocarcinomas was evaluated by IHC in 50 curatively resected tumors.

The incidence of HER2/neu over expression is gastric adenocarcinoma in our study (10.0%) is identical to that reported in other large studies in the world. Similarly, studies and detailed reports from India are also available in indexed literature.^[2,3] A lack of interest in pursuing HER2/neu status in advantage gastric cancer is possibly because trastuzumab is still out of reach of a large majority of Indian patients who do not have any kind of medical insurance to cover the cost of such treatment.^[9] Nevertheless, this study brings out certain facts discussed as follows that has to be confirmed on a bigger subset of cases in future studies.

HER2/neu expression in gastric cancers varies from 7 to 44%. But most of the studies included biopsies as well as gastrectomies. Higher rates of HER2/neu positivity in biopsies may have been due to larger number of cases in the study when compared to gastrectomy in the other studies. Another reason may be better fixation of biopsy specimens as suggested by Ruschoff J and coworkers.^[10] Geographic and ethnic heterogeneity of solid tumors may also explain the differences of HER2/neu overexpression in various studies.

The results of present study matches with the study done by Yano and coworkers and Lordick and coworkers,^[11] and also with TOGA trial 12, Which reported HER2/neu expression Positivity 22.1% overall and

10.4% in resected samples. In a study by Ling Shan and co-workers, out of 1463 patients, HER2/neu overexpression was noted in 9.8% while 14.4% and 75.8% were weakly positive and negative, respectively.^[13]

It should be pointed out that the present study was purely based on IHC, and we have considered 2+ as weak positive. The 2+ results should be further be evaluated by CISH/FISH methods according to the current recommendations.^[10]

One of the drawbacks of our study is that we were not able to confirm IHC 1+and 2+cases by FISH analysis due to economic constraints, and hence, our HER2/neu positivity rate may actually be lower. HER2/neu studies have shown excellent concordance rate (>95%) between IHC and FISH in IHC 0 and IHC 3+ cases, suggesting that these IHC scores may not require routine FISH confirmation.in the ToGA trial, 26% of the Score 2+ were Fish positive for HER2/neu overexpression, while in a Chinese cohort study, 28.8% of equivocal turned positive upon FISH evaluation.^[13]

Another pitfall of IHC that we noticed was that normal foveolar epithelium showed nonspecific cytoplasmic staining. But there was clear distinction between normal epithelium and tumour cells and, hence, this did not interfere with the interpretation. But one needs to be careful during interpretation of HER2/neu staining. Besides, there have been reports of truncated or secreted forms of the HER2/neu receptor that are not anchored in the cell membrane and that can be detected immunohistochemically as a Cytoplasmic staining pattern.^[8]

Intense Cytoplasmic positivity (probably related to Cytoplasmic mucin) in the tumour cells though considered non – specific in the study, needed FISH confirmation because strong cytoplasmic staining can mask membranous positivity.^[14] HER2/neu expression was fairly distributed throughout the age group .There was no statistically significant association ($P=0.972$) between HER2/neu expression and age, as was also noted in other studies.^[4,13] HER2/neu expression was 14.2% (2/35) in males and 0% in females, showing no significant association of HER2/neu expression and gender. ($p=0.333$) similar trend was observed in some other studies.^[4]

In moderately differentiated and poorly differentiated tumors, score 3+ positivity was seen one case for each grade (1/16, 6.2%) and (1/28, 3.5%) respectively, compared to well differentiated tumors which showed (1/ 6, 16.6%) with Score 2 + positivity. So the well and moderately differentiated have shown higher percentage of HER2/neu positivity. This result is observed in our study which correlated with other studies like Sekaran et al,^[2] Rajagopal et al,^[3] and Yan et al.^[11] Many studies exhibit high HER2/neu expression in GEJ cancers compared to gastric cancers. HER2/neu overexpression may even be higher in esophageal cancers.^[8,9] All the tumors that showed HER2/neu positivity in our study were in the antro-pyloric area.

In our study, 35/50 patients had intestinal type gastric cancer and 4/35 (11.4 %) of these were HER2/neu positive compared to 1/15 (8.3%) with diffuse-type cancer. Thus HER2/neu expression was more in intestinal –type cancer in our study ($p=0.083$).^[15]

CONCLUSION

Our study found HER2/neu over expression of 10.0% in gastric cancers similar to most studies in India and the rest of the world. Additional research is required to explore role of HER2/neu as an independent prognostic factor and to confirm any existing geographic variation.

All advanced gastric and GEJ carcinomas should undergoHER2/neu status evaluation. Cases with HER2/neu equivocal results in IHC should undergo FISH analysis to confirm HER2/neu status. Further studies with cohorts need to be conducted to provide more clarity on prevalence of HER2/neu over expression.

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