ORIGINAL RESEARCH PAPER

Volume-9 | Issue-2 | February-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY OF HER2/NEU AND Ki-67 EXPRESSION IN GASTRIC AND ESOPHAGO-GASTRIC JUNCTION ADENOCARCINOMA AND THEIR CORRELATION WITH GRADE AND STAGE

Pathology

Dr. Piyali Pramanik Post Graduate trainee, Department of Pathology, Nil RatanSircar Medical College, Kolkata

Dr. Ranu Sarkar* Professor and Head, Department of Pathology, Nil RatanSircar Medical College, Kolkata *Corresponding Author

Dr. Moumita Maity Associate Professor, Department of Pathology, Nil RatanSircar Medical College, Kolkata

ABSTRACT

INTRODUCTION: Gastric cancer is one of the most aggressive malignancies with an extremely poor prognosis. It accounts for 5.7% of cancer worldwide with a mortality rate of 8.2% . Surgical resection is the mainstay of therapy for gastric and esophago-gastric junction(EGJ)carcinomas followed by chemotherapy. However, most patients are diagnosed in an unresectable stage with decrease in survival rates. New therapeutic strategies like targeted therapy may help to improve prognosis. Patients with HER2/neu overexpression benefit from transtuzumab therapy. It is therefore essential to determine the HER2/neu status of tumors.

OBJECTIVE OF RESEARCH: To study the expression of HER2/neu and Ki67 index in histologically diagnosed cases of gastric and EGJ adenocarcinoma and their clinicopathological correlation.

METHODOLOGY :A prospective, observational, non- interventional study was done in the Department of Pathology in collaboration with Department of General Surgery,NRS Medical college, Kolkata in a duration between 1st January 2018 to 30th June 2019.All the total and partial gastrectomy specimen& specimen of endoscopic biopsy were processed in the Department of Pathology for Histopathological examination and IHC study.

RESULTS: Among 52 cases of gastric and EGJ adenocarcinoma, majority were males (69.2%) and antrum (63.5%) being the most common site. HER2 expression was observed in 23.0% of cases and statistically significant correlation was found with intestinal type ($p<0.0001$), tumour grade ($p= 0.0152$) and location of the tumor ($p= 0.028$). High Ki-67 index was observed in 67.3% of gastric and EGJ adenocarcinoma having significant correlation with tumor grade ($p<0.0001$)and stage($p<0.001$).

CONCLUSION: Intestinal-type and moderately differentiated gastric cancers predominantly showed HER2 neu positivity and also high Ki67 proliferation index. So, these patients may be considered for targeted therapy using Trastuzumab in combination with chemotherapy

KEYWORDS

gastric and EGJ adenocarcinoma, HER2/neu, Ki67

INTRODUCTION:

Gastric cancer is one of the most aggressive malignancies with an extremely poor prognosis and it accounts for 5.7% of cancer worldwide with a mortality rate of 8.2%[1]. It was responsible for over 1,000,000 new cases in 2018 and an estimated 783,000 deaths (equating to 1 in every 12 deaths globally), making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death[1]. Incidence rates of esophago-gastric junction (EGJ) adenocarcinomas also showed a marked increase in incidence in recent years[2]. Surgical resection is the mainstay of treatment in early stage cancers, but, most patients are diagnosed at an advanced stage which are often unresectable.EGJ cancer spread to the surrounding important structure at an early stage. The survival rate of patients with advanced resectable gastric and EGJ cancers, however remains poor despite chemotherapy. So, early diagnosis of the tumor and institution of molecular targeted therapies can help to prevent recurrence, metastasis and improve patient survival[3,4].

Patients with advanced gastric or EGJ adenocarcinoma are studied in order to verify whether the tumors demonstrated an overexpression of HER2 protein, as detected by immunohistochemistry (IHC).The characteristics of histological type, grade, stage and Ki67 expression are also analyzed.

HER2/neu acts as an oncogene and helps in uncontrolled proliferation of cells[5]. Amplification of HER2/neu oncogene has become an important biomarker for identifying patients who respond to HER2/neu targeting therapy using humanized monoclonal antibody.The randomized ToGA study revealed a 26% reduction in the risk of mortality when Transtuzumab was added to the chemotherapy regime for treating advanced gastric carcinoma[6].

Some studies indicate that the rate of Ki67 before neoadjuvent chemotherapy is a strong predictor of efficacy of the therapy. It is found that high level of Ki67 showed complete response and after neoadjuvant chemothaerapy, lower value of Ki67 indicates a better prognosis[7].A high Ki-67 was seen predominantly inHer2-neu positive cases. Her2-neu negative cases showed moderate to low Ki-67. Her2-neu positivity is strongly associated with high Ki-67 proliferation index[8].

The current study intends to categorically assess the overall incidence of different subtype of gastric and EGJ adenocarcinoma, HER2/neu expression and Ki67 index by immuno-histochemistry techniques on diagnosed case of adenocarcinoma and to study the correlation of HER2/neu and Ki67 expression with histological type, grade and stage. The study also aimed to determine whether the expression of HER2/neu was associated with more aggressive behavior and a poorer outcome by its correlation with Ki67 expression. This study shall moreover help the clinicians to plan out the further line of treatment specially in advanced cases.

OBJECTIVE OF RESEARCH

1. To diagnose cases of gastric and esophago-gastric junction (EGJ)neoplasm by histological examination and to determine histological type, grade and stage.
2. To analyse the expression of HER2/neu and Ki67 in the diagnosed cases of gastric adenocarcinoma including EGJ neoplasm by IHC.
3. To study the correlation of HER2/neu and Ki67 index with different clinicopathological parameter.
4. And to determine whether there is any correlation between HER2/neu expression and Ki67 index.

METHODOLOGY

An institutional based cross sectional, observational study was conducted in a tertiary care center of West Bengal from January 2018 to June 2019 in the department of pathology in collaboration with department of General Surgery, NRS Medical College and Hospital.All the total and partial gastrectomy specimen& specimen of endoscopic biopsy were included in the study with the exclusion of cases with history of chemotherapy and the cases diagnosed other than adenocarcinoma. A total of 52 cases of diagnosed gastric and EGJ adenocarcinoma which include 46 resected specimen and 6 endoscopic biopsy specimen. Census method of sampling was used. Data was collected using a pre-designed, pre-tested semi structured schedule on dependent variables like HER2/neu and Ki67 expression and independent variables like clinic-pathological profile including age at presentation, sex, histological type, grade, stage and other relevant parameters. Data was collected by observations, record review and laboratory techniques including histopathology and

immunohistochemistry.

Histopathology:
All tissue samples were collected in 10% buffered formalin and processed for routine histopathological examination. 5 µm thick sections from formalin fixed paraffin embedded blocks were cut and stained with hematoxylin and eosin for histopathological diagnosis of tumor type, grade and stage.

Immunohistochemistry (IHC):
For IHC staining 3 µm thick sections from formalin fixed paraffin embrdded tissues were taken on poly L Lysine coated slides. IHC was done using HER2/neu and Ki67 antibody and the steps mentioned in the kit were followed.

Scoring of HER-2/Neu IHC: The samples were analyzed using standard criteria for HER-2/neu positivity. Positivity was assessed as brown cell membrane staining of malignant cells. All slides were scored independently by the 3 authors according to the Gastric Cancer Scoring System (GCSS) for Surgical Specimens as follows[9]. Score 0/negative: No staining or membrane staining in <10% of invasive tumor cells. Score 1+/negative: Faint/barely perceptible membrane staining in ≥10% of invasive tumor cells; cells are only stained in part of their membranes. Score 2+/equivocal: Weak to moderate complete or basolateral membrane staining in ≥10% of invasive tumor cells. Score 3+/positive: Moderate to strong complete or basolateral membrane staining in ≥10% of invasive tumor cells. All the cases that were given a score of 2+ were further assessed using FISH.

Scoring of Ki-67 IHC: The samples were analyzed following the recommendations from the International Ki-67 in Breast Cancer Working Group[10] ; whereby positive Ki-67 staining was defined as only positive nuclear staining counting of at least 1000 nuclei at high-power (×40 objective), regardless of the staining intensity. For proper grouping of results, cases were then categorized into: GCs with high Ki-67 score (>20%) and GCs with low Ki-67 score (≤20%).

RESULTS:
Clinicopathological findings:
The study group consisted of 52 cases of gastric and EGJ adenocarcinoma which include 46 resected specimen and 6 biopsy specimen.In this study the mean age of the patients was 55.30±12.71 years with a range of 20 - 81 years and there was a male preponderance of cases (69.2%) with M:F ratio of 2.3:1.The most common location of carcinoma was antrum(63.5%) followed by body(25.0%) . EGJ adenocarcinoma and carcinoma of the fundus and body constitute 5.8% each and among the macroscopic types, ulcerative tumors were the most common (63.5%)According to Lauren's histological type diffuse (poorly cohesive) and intestinal adenocarcinoma constituted of 57.7% and 42.3% respectively and most common tumor was of poorly differentiated type (51.9%) followed by moderately differentiated (40.4%)type.With regard to TNM staging the majority of patients(71.1%) were at stage III and IV. Lymphovascular invasion was detected in 54.3% of the cases whereas lymph node metastasis was present in 63.5% of cases.

HER2/neu expression and its correlation with clinicopathological parameter:
Out of 52 cases, HER2/Neu was positive in 12 cases(23%) only.

Positive HER2/neu expression was significantly associated with tumor location(p=0.028), intestinal histological type(p=0.0001) and moderately differentiated carcinoma according to histological grade (p=0.0152) (Table 1).

In the present study we also found positive correlation between HER2/neu expression with male sex, higher tumor stage and cases with lymph node metastasis without any statistical significance.

Ki67 index and its correlation with clinicopathological parameter:
Among the total 52 cases high Ki67 proliferation index was seen in 35 cases(74.2%).

There were statistically significant association between high Ki67 proliferation index with high grade(p<0.0001) (Table 6, Graph 6) and stage (p<0.001) of the tumor(Table 7, Graph 7).There was a strong positive correlation between Ki67 index and diffuse (poorly cohesive) type ofadenocarcinoma(p=0.09) (Table 2).

High Ki67 index was also observed in patients with age>50 years, male sex and patient with lymph node metastasis, but these association was statistically insignificant.

Correlation between HER2/neu expression and Ki67 proliferation index:
In the present study, most of the HER2/Neu positive cases (83.3%) showed high Ki67 index, but the overall HER2/Neu positivity failed to show significant correlation with Ki67 proliferation index (p=0.17) (Table 3).

TABLES, GRAPHS & FIGURES:
Table-1: Association between Her2/neu of the patients with different clinicopathological parameters

Parameters	Status of Her2/neu		Test significance (p value)
	Positive(%)	Negative(%)	
Age			0.70
≤50	5(29.4)	12(70.6)	
>50	7(20.0)	28(80.0)	
Sex			0.62
male	9(25.0)	27(75.0)	
female	3(18.8)	13(81.2)	
Location of tumor			0.028
EGJ	2(66.7)	1(33.3)	
Fundus	2(66.7)	1(33.3)	
Body	4(30.8)	9(69.2)	
Antrum	4(12.1)	29(87.9)	
Histological type			<0.0001
intestinal	11(50.0)	11(50.0)	
diffuse	1(3.3)	29(96.7)	
Histological grade			<0.0152
well differentiated			
moderately	1(25.0)	3(75.0)	
differentiated	9(42.9)	12(57.3)	
Poorly differentiated	2(7.4)	25(92.6)	
Pathologic tumor stage	5(33.3)	10(66.7	0.39
T1, T2			
T3, T4	7(18.9)	30(81.1	
Lymph node metastasis			0.473
Present	8(25)	24(75)	
Absent	4(20)	16(80)	

Table-2: Association between Ki67 status of the patients with different clinicopathological parameters

Parameters	Status of Ki67		Test significance (p value)
	High (%)	Low (%)	
Age			
≤50	9(52.9)	8(47.1)	0.33
>50	26(74.3)	9(25.7)	
Sex			
male	23(63.9)	13(36.1)	0.43
female	12(75.0)	4(25.0)	
Histological type			
intestinal	12(54.5)	10(45.5)	0.09
diffuse	23(76.7)	7(23.3)	
Histological grade			
well differentiated	1(25.0)	3(75.0)	<0.0001
moderately differentiated	8(38.1)	13(61.9)	
Poorly differentiated	26(96.3)	1(3.7)	
Pathologic tumor stage			
T1, T2	10(66.7)	5(33.3)	<0.001
T3, T4	25(67.5)	12(32.5)	
Lymph node metastasis			
Present	21(65.6)	11(34.4)	0.74
Absent	14(70.0)	6(30.0)	

Table-3: Association between status of Her2/neu and status of Ki67 of the patients

Status of Her2/neu	Status of Ki67		TOTAL
	High	Low	
Positive	10	2	12
Row %	83.3	16.7	100.0
Negative	25	15	40
Row %	62.5	37.5	100.0

TOTAL	35	17	52
Row %	67.3	32.7	100.0

PHOTOMICROGRAPHS;

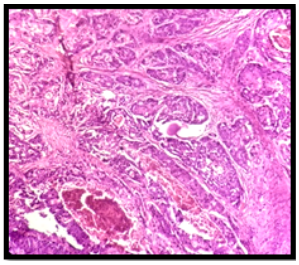

Fig:Moderately Differentiated Adenocarcinoma(H&E)

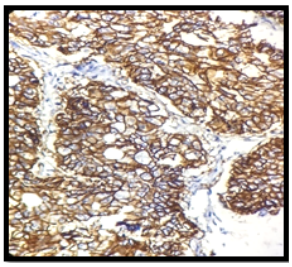

Fig: Moderately differentiated adenocarcinoma(IHC with HER2/neu) showing 3+positivity

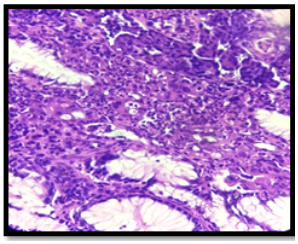

Fig: Poorly differentiated intestinal adenocarcinoma (H&E)

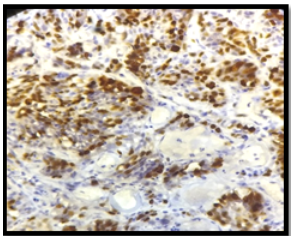

Fig: Poorly differentiated intestinal adenocarcinoma (IHC with Ki67) showing high Ki67 index

DISCUSSION:

In the present study, out of 52 cases of gastric and EGJ adenocarcinoma, age of the patients ranged from 20-81 years with a mean age of 55.30±12.71 years consistent with the study of H.Amrani H.J. et al[11] ,Saba EI-Gendi et al[12].There was a male preponderance(69.2%) with male to female ratio being 2.3:1.0, which correlate with the study of Indu Rajago Pal et al[13] ,Daniela Lazar et al[14].

In our study the most common location of carcinoma was antrum(63.5%)consistent with the previous study done by Saba EI-Gendi et al[12] ,Indu Rajago Pal et al[13].

Among the Lauren histological type, this study population constituted 57.7% of diffuse gastric carcinoma while intestinal type constitute 42.3% cases. Our study showed a decreasing trend of intestinal type adenocarcinoma and is from the previous study done by Sunitha et al[15],Ayesha Ahmed et al[16] and Saha et al[17]. It may be due to our small study population or decreased prevalence of H. pylori and improvements in the preservation and storage of foods.In the present study, most of the cases were poorly differentiated (51.9%), followed by moderately differentiated type(40.4%) and least number of well differentiated type(7.7%) consistent with the study done by Begnami et al[18] ,Calik et al[19].

With regard to TNM staging the majority of patients(71.1%) presented at a higher stage (III and IV) and this is corcordant with Ola A Harb et al[20] ,H.Amrani H.J. et al[11]. In the present study most of the patients had lymph node metastasis (63.5%) which is also concordant with Saba EI-Gendi et al[12] ,Tarek GHARSALLI et al[21].

In the present study, out of total 52 cases of gastric and EGJ adenocarcinoma, 12 cases showed HER2/neu positivity(23.1%) cases. There is a wide range of HER2/neu positivity in various studies done previously.However, our study matches with the findings of Indu RajagoPal et al[13] Rajat Jagani et al[22] and Piyali Ghosh et at al[23].

In this study it is seen that Her2/Neu expression was more common individuals greater than 50 years of age and male sex. However , no statistically significant correlation found between Her2/neu expression with different age group and gender, consisted with the study of Sunitha et al[15].

In our study positive Her2/neu was mostly prevalent in carcinoma of EGJ (66.7%) and Fundus and body (66.7%) which were significantly higher ($p<0.0001$) than antral location(12.1%) and it is consistent with Indu RajagoPal et al[13] ,Rajat Jagani et al[22].

Out of the 12 HER2/neu positive cases in our study , 11 cases were of intestinal type whereas only 1 case of diffuse type showed HER2 positivity which is concordant with the study of M Tanner et al[3] Sekaran et al[24].

Among the degree of differentiation, moderately differentiated adenocarcinoma showed maximum (42.9%) HER2/ Neu positivity followed by well differentiated and poorly differentiated type, consistent with the study of Indu RajagoPal et al[13]

In the present study, no significant correlation found between positive HER2/neu expression with stage of the tumor and lymph node metastasis which is consistent with Indu RajagoPal et al[13]Tarek GHARSALLI et al[21]

In this study out of total 52 cases, 67.3% cases showed high Ki67 proliferation index which is comparable with the previous study. The present study showed high Ki67 index was positively correlated with older age of the patients and male gender but without any statistical significance, corcordant with the study done by Daniella lazar et al[14]

With regard to Lauren's histological type, 65.7% of the diffuse gastric carcinoma showed high Ki67 proliferation index which is consistent with Saba EI-Gendi et al[12]

In the present study statistically significant correlation found between high Ki67 index with high grade and stage of the tumor consistent with the study done by Daniella lazar et al[14]

In the present study,no statistically significant($p=0.74$) found between Ki67 index with lymph node metastasis, concordant with the study of H. Ambrani HJ et al[11].

Lastly we also aimed to determine whether the expression of HER2/neu is associated with a more aggressive behaviour by its correlation with Ki67 index. In the present study, 10 out of 12, HER2/neu positive cases showed high Ki67 proliferation index , but the overall HER2/Neu positivity did not correlate significantly with Ki67 proliferation index.($p=0.17$) correlated with the study of Saba EI-Gendi et al[12]

CONCLUSION:

In conclusion, 23% of gastric and GEJ cases showed HER-2/neu positivity. Tumors showing HER-2/neu positivity and high Ki67 index were associated with higher grade and T-stage and most of the HER2/neu positive cases(83.3%) showed high Ki67 index, implying a more aggressive behavior. HER2/Neu positive gastric and EGJ adenocarcinoma patients when given treatment with targeted therapy(Transtuzumab) have a better response with improved survival. Further studies on a larger number of cases with follow up of the patients receiving targeted therapy(Transtuzumab) will contribute to a better knowledge regarding the utility of HER2/Neu and Ki67 immunohistochemistry routinely on gastric and EGJ adenocarcinoma cases.

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