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ANALYSIS OF EXPRESSION OF HER-2 RECEPTOR IN GASTRIC AND GASTROESOPHAGEAL JUNCTION ADENOCARCINOMAS

Oncology

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ABSTRACT

Background: Gastric and gastroesophageal junction adenocarcinomas (GEAs) is associated with significant morbidity and mortality due to early metastasis. HER2 receptor of the RAS-RAF-MAPK pathway and PI3K signalling pathway has been targeted successfully in breast carcinoma. The ToGA trial shows significant improvement in survival treated with trastzumab in patients with advanced or metastatic gastric and gastroesophal junction adenocarcinomas. We analyse the expression of HER2 receptor in patients with metastatic GEAs. **Methods:** This observational study was conducted at Indraprastha Apollo hospitals, New Delhi between july 2017 to june 2019 and included 70 patients who presented with metastatic gastroesophageal adenocarcinomas and to analyse if there is any correlation with respect to age, gender, location of the tumour, histological grade and Lauren classification. **Results:** Out of 70 patients, 9 patients had overexpression of HER2 (12.8%) by immunohistochemistry. HER2 overexpression is not significantly different with respect to age, gender, location of the tumour, histological grade and Lauren classification. **Conclusion:** HER2 overexpression in gastric and gastroesophageal junction adenocarcinomas is seen in relatively low proportion of our patients and no specific patient and tumour characteristics could be identified where HER2 overexpression is more likely.

KEYWORDS

HER2, Gastric cancer, GEJ, IHC

INTRODUCTION

Tumours arising from gastroesophageal junction is often ambiguous and could not be clearly defined as esophageal or gastric in origin and are therefore called as gastroesophageal junction (GEJ)tumours. Gastric cancer(GC) and GEJ adenocarcinomas can be collectively referred as Gastroesophageal adenocarcinomas(GEAs). GEAs are often diagnosed in a locally advanced or metastatic stage when tumour is unresectable. The response to current chemotherapeutic agents is poor with a response rate of 10-60% and survival of patients on palliative chemotherapy remains poor [1]. In the search for new therapeutic agents, HER2 protein expression of the epidermal growth factor appears promising. HER2 is encoded by a gene located in chromosome 17q21 known as ERBB2. It encodes the 185 KDa receptor receptor HER which has an extracellular ligand binding domain. HER2 overexpression is a key driver of oncogenesis through its association with tumour cell proliferation largely mediated through the RAS-RAF-MAPK pathway and it inhibits cell death through the PI3K-Akt-mTOR pathway. The ToGA phase III randomized controlled trial revealed that combination treatment with trastuzumab and chemotherapy significantly improved survival in patients with locally advanced and metastatic GEAs with HER2 overexpression[2]. Median overall survival was 13.8 months in those assigned to trastuzumab plus chemotherapy compared with 11.1 months in those assigned to chemotherapy alone.This study shows improved overall survival in locally advanced and metastatic gastroesophageal adenocarcinomas [HR- 0.74]. Thus trastuzumab was recently approved for the treatment of metastatic adenocarcinomas of the gastric and GEJ origin in many countries.

MATERIALS AND METHODS

This was a prospective observational study conducted at Indraprastha Apollo hospitals, New Delhi and included 70 Locally advanced and metastatic Gastric and GEJ cancers patients presented as either index case or referred to our institute between July 2017 to June 2019. They were screened and those fulfilling the inclusion/exclusion criteria were taken up for the study. Study population were selected from Gastric and gastroesophageal junction adenocarcinomas patient, proven histologically, in our institute. The primary end point was to find out the percentage of Gastric and gastroesophageal junction adenocarcinomas over-expressing HER2 receptor. The secondary end points of the study was to find out the effect, if any, of location of the tumour, age, gender of the patient, stage and histological grades of the tumour and Lauren classification on HER2 over-expression.

Inclusion Criteria

- Patients with Gastric and GEJ adenocarcinoma from whom tissue has been obtained either by endoscopic biopsy or by surgical excision.
- Those in whom a complete staging workup could be achieved
- Patient older than 18 years of age.
- Patient of either sex

Exclusion Criteria

- Those who had received any neoadjuvant treatment before obtaining the tissue.
- Those where tissue sample obtained was incomplete/inadequate
- Those in whom a complete Stage workup was not possible.
- Tumour histology other than adenocarcinoma.

Statistical Analysis

Data were collected and entered in Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) after checking for correctness and consistency. Data were analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). Data were described using mean, median, and percentages. A test of significance was performed using Chi square/ fisher exact test as required. A probability value of less than 0.05 was taken as significant.

RESULTS

Tumor samples from 70 GEAs patients was evaluated by immunohistochemistry (IHC) analysis. The patients clinicopathological characteristics were listed in table 1. The median age was 59.27 years (range 26-86 years) with a male predominance (70%). Of all patients there was 42 gastric and 28 GEJ adenocarcinomas. There were 46 patients (65.7%) of intestinal type 17 (24.3%) of diffuse type and 7 (10%)of mixed type by Lauren classification. Tumours were well differentiated in 11 (15.7%), moderately differentiated in 34 (48.6%) and poorly differentiated in 25 (35.7%) patients. Of the 70 patients 9(12.9%) patients were scored as HER2 overexpression (IHC-3+) and 61 patient didn`t overexpress HER2 (IHC-2+,1+,0). HER2 was overexpressed (IHC-3+) in 5(11.9%) gastric cancer patients and 4 (14.3%) of GEJ adenocarcinomas. HER2 was negative in 88.1% (37/42) of gastric cancer patients and 85.7% (4/28) of GEJ adenocarcinomas. There were no significant difference in HER2 overexpression between gastric cancer and GEJ adenocarcinomas (11.9 % Vs 14.3%, P=1.000). HER2 overexpression was not associated with any type by Lauren classification (P=1.00, 13% in intestinal type, 11.8% in diffuse type, 14.3% in mixed type). No correlation was observed between HER2 overexpression in terms of histological grade of tumours by WHO classification (p=0.632), age and sex.

Table No. 1; Clinicopathological Characteristics And Her2 Overexpression

Clinicopathological features	Number of patients	HER2 overexpression (%)	HER2 negative (%)	P value

1. Age at diagnosis	i) <60 years	31	2(6.5)	29(93.5)	0.215
	ii) ≥60 years	39	7(17.9)	32(82.1)	
2. Sex	Male	49	6(12.3)	43(87.7)	1.000
	Female	21	3(14.3)	18(85.7)	
3. Tumour location	GC	42	5(11.9)	37(88.1)	1.000
	GEJ	28	4(14.3)	24(85.7)	
4. Histological grade	Well differentiated	11	1(9.1)	10(90.9)	0.632
	Moderately differentiated	34	6(17.6)	28(82.4)	
	Poorly differentiated	25	2(8)	23(92)	
5.Tumour subtype (Lauren classification)	Intestinal type	46	6(13)	40(87)	1.000
	Diffuse type	17	2(11.8)	15(88.2)	
	Mixed	7	1(14.3)	6(85.7)	
6. Stage	Stage IV(metastatic)	39	5(12.8)	34(87.2)	1.000
	Locally advanced	31	4(12.9)	27(87.1)	

DISCUSSION

In our study, total number of GEAs patient over-expressing HER2 receptor by IHC (score 3+) was 9 (12.86%). There is wide range in the reported incidence of HER2 receptor over-expression in GEAs. A summary of the few Indian and important previous study in literature is given in table no 2. This difference is largely due to heterogenous population, different methodologies or scoring system used for interpreting the results. For example few of the studies have included IHC score of 2+ in addition to 3+ as positive result [3,4,5] while others included only IHC 3+[6,7,8]. Some studies in fact reported the results of HER2 expression in cohorts which include all the GC and GEJ cancers while others include only GC. Another issue in the differences in the patient cohorts studied is that some study included only early gastric cancer while other included both the early and advanced gastric adenocarcinomas. Such differences in patient selection across the studies have to be factored in interpreting the result.

Table No 2: Summary Of Results Of Studies Of Her2 Expression In GEAs

Author	Types of cancer	Criteria for HER2 positivity	No of patients	Percentage of HER2 positivity	Clinico-pathologic relation
Park et al [3]	GC	IHC 2+, 3+	182	15.9%	HER 2 positivity more common in intestinal type. HER2 overexpression associated with poor mean survival rates (922 vs 3243 days) and 5-year survival rates (21.4%vs 63.0%; P<0.05)
Tewari M et al [4]	GC, GEJ	IHC 2+, 3+	70	21.4%	HER2 positivity more common in intestinal type (45% Vs 12%, significant)
Xie et al. [5]	GC	IHC 2+, 3+	218	18.8%	No relationship with clinicopathological parameters. HER 2/neu expression was correlated with poorer overall survival (p<0.001).HER 2 expression was a significant independent prognostic predictor of gastric cancer (p<0.001), and was associated with poor survival in gastric cancer patients
Rajgopal I et al [6]	GC, GEJ	IHC 3+	60	26.7%	GEJ showed HER2 expression in 45.5% as opposed to 22.4% in GC
Shan L et al [7]	GC, GEJ	IHC 3+	1463	9.8%	HER2 overexpression (3+) were more frequently observed in GEJ cancer cases, in the intestinal type, and in the well or moderately differentiated type (P=0.003, 0.000, and 0.000, respectively).
Yoshida et al. [8]	GC	IHC 3+	207	17%	Concordance rates between IHC and FISH was 90.9 % in surgically resected tumors and 90.2 % in biopsy specimens. In IHC 2+ cases, the rate of HER2 gene amplification was 56%and 38 % in surgically resected tumors and biopsy specimens respectively.
Aditi R et al [11]	GC	IHC 3+, IHC 2+ and FISH+	58	27.6%	HER2 positivity more common in intestinal type (34.9 % Vs 6.7%, significant)
Cutsem EV et al [10]	GC, GEJ	IHC 3+, any IHC with FISH +	3665	22.1%	HER2 Overexpression more in intestinal histology than diffuse histology (31.8 % vs. 6.1 %, respectively) ,GEJ than GC (32.2 % vs. 21.4 %). Biopsy specimens have mor HER2 overexpression compared with surgical specimens (23.2 % vs. 19.7 %, respectively)

We used the ASCO/CAP scoring criteria established for gastroesophageal adenocarcinoma for reporting the IHC results[9] . Fluorescence in situ hybridization(FISH) was not performed in cases with IHC score of 2+ for financial reasons and were considered HER2 negative for the study purposes. we limit a positive result of overexpression to IHC score of 3+ only. Another possible reason for wide range in HER2 positivity could be due to method of tissue obtained for testing. For example in the study by Cutsem EV et al [10] there was also a slightly higher rate of HER2 overexpression/ amplification in biopsy specimens compared with surgical specimens (23.2 % vs. 19.7 %, respectively). Aditi R et al [11] found 34.1% of biopsy specimen and 11.8% of surgical specimen positive for HER2. Davidson and Pai have suggested 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 [12]. 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. They proposed the progressive loss of activity of labile macromolecules after the surgical interruption of blood flow leading to tissue ischemia, acidosis, and enzymatic degradation.

The mean age of our study population is 59.27 year with age ranging from 26 to 86 years (mean ages for men was 60.57 and 56.26 for women). Majority of the patients studied were in the age groups of 50-

70 years (65.7% of the population). This reflects the higher incidence of GEAs in older age group. A similar result is reported from studies across the globe. The percentage of HER2 overexpression when divided between those younger than 60 year and those older, did not show any statistical significance (p=0.215). 2 out of 31 (6.5%) cases of GEAs younger than 60 year of age were positive for HER2 while 7of 39 cases (17.9%) ≥ 60 year of age were showing HER overexpression.

There were 49 men and 21 women constituting 70% and 30% of the population studied. A higher incidence GEAs amongst the male explained the higher proportion of men to women in our study cohorts. Men to women ratio was 3:1 in the study reported by Shan L et al [7] and men constituted 71.4 % in the study population reported by Tewari M et al [4]. There was no statistically significant correlation of gender with HER2 overexpression (p= 1.000). 12.24 % of men and 14.28 % of women in our study has HER2 overexpression.

HER2 positivity was found in 14.3% of GEJ and 11.9% of gastric cancer in our study. Our findings are consistant with a large study reported by Shan L et al [7] in which they reported a HER 2 positivity of 14.6% and 7% in GEJ and GC respectively. Reported rates of HER2 overexpression in gastroesophageal cancer vary widely (4–53%) due to small sample sizes, differences in patient populations and methodological and scoring differences between studies. In addition, differences between HER2 overexpression in European and Asian/South-American populations (22–28% versus 3–15%, respectively) have been reported by some studies [13,14]. Table no 3 shows the HER2 positivity rates in various studies according to location of the primary tumour and histological types.

Table no 3: Comparison of HER2 Overexpression in GEAs Reported by Various Authors Based On Tumor Location And Histological Type With Our Study

Author	Histologic type		Location of tumour	
	Intestinal (%)	Diffuse (%)	GEJ (%)	GC (%)
Tewari M et al [4]	45	12		
Rajgopal I et al [6]	32.7	0	45.5	22.4
Shan L et al. [7]	16.8	2.3	45.5	22.4
Cutsem Ev et al [10]	31.8	6.1	32.2	21.4
Tanner et al.[15]	21.5	2	24	12
Gravalos et al.[16]	16	7	25	9.5
Lordick et al., [17]	34	6	32	18
Our study	13	11.8	14.3	11.9

In the literature, the definition of early gastric cancer (EGC) is clear, however, there is heterogeneity in the definition of advanced gastric cancer. EGC refers to those adenocarcinomas with growth that is limited to the mucosa or submucosa, independent of regional lymph node (LN) metastases. For our study purposes locally advanced gastric cancer is defined as : T3–4 any N or T≤2 N+. 39 cases in this study were Stage IV disease and 31 cases were locally advanced. HER2 positive cases were seen in 5 of 39 (12.8 %) Stage IV cancers and 4 of 31 (12.9 %) locally advanced cancers and was not statistically significant (p=1.000). Similarly there was no statistically significant association of HER2 positivity with the grade of the tumour (p=0.632). 1 out of 11 (9.1%) well differentiated tumour showed positive result while 6 out of 34 (17.6%) moderately differentiated and 2 out of 25 (8%) were poorly differentiated .

In the present study , HER2 positivity in intestinal, diffuse and mixed subtype was observed in 13%, 11.8% and 14.3% respectively and not statistically significant (p= 1.000). Recent studies have reported the intestinal type (16-34%) has higher prevalence of HER2 overexpression compared to diffuse type (2-7%) [6]. Most studies have found an increased incidence of HER2 positivity with the intestinal type while Tiwari M et al [4] found a HER2 positivity rate of 45% and 12% in the intestinal and diffuse type respectively in their study. The reasons for the selective overexpression of HER2 in intestinal type of gastric carcinomas are complex. The association of HER2 with a specific histologic Tumour type suggests that intestinal and diffuse types develop along different molecular pathways.

The correlation of HER overexpression with stage and grades of tumour have mixed results in previous reports; some reporting direct correlation with more advancing stage and better differentiation of tumours while other failed to show any such relationships. Shan L et al [7] in their study on 1463 cases of GEAs patient found a statistically significant correlation of HER expression with grades of tumour. HER2 overexpression was highest amongst moderately differentiated tumours (20.1%) followed by well differentiated (16%) and poorly differentiated histology (6.1%). In our study HER2 positivity was highest amongst the moderately differentiated histology with 17.6% of them positive by IHC. These were followed closely by well differentiated (9.1%) and poorly differentiated tumours (8%) which was however not statistically significant (p=0.632). Rajgopal I et al [6] found a statistically significant HER2 overexpression in patients with the well and moderately differentiated histology. However, Aditi R et al [11] have reported a trend of HER2 positivity towards more advanced stage and more undifferentiated tumour histology. These conflicting data may be due to different sample sizes and the low prevalence of HER2 in GC and GEJ adenocarcinoma. In addition, varying methods of evaluation and scoring schemes with different cut-points were used before the establishment of a standard guideline for assessing HER2 expression. Perhaps with the current consistent guidelines for HER2 assessment in this disease, future studies will provide more clarity regarding this issue.

There are substantial number of studies demonstrating both association and no association between HER2 overexpression including histologic grade, Lauren classification, stage of tumour and presence of metastasis. Despite the overall impression that well differentiated and intestinal histology seem to have stronger staining intensities that are emphasized at the cell membranes, the heterogeneity of population studied, different criteria for HER2 positivity and different disease composition makes it impossible to provide definitive conclusion from the literature on the association of HER2 overexpression with these clinicopathologic markers. In the present study also, no statistically significant correlation was found for HER2 with various clinicopathologic markers.

One of the drawbacks of our study is that we were not able to confirm IHC 2+ cases by FISH. FISH test was not included due to financial reasons and hence only IHC 3+ was taken as positive result. Secondly, the source of tissue was not same. Higher rate of HER2 positivity tested in biopsy tissues has been shown in various studies [10,11]. Moreover reliable separation of IHC 1+ and IHC 2+ patterns is challenging in small biopsy specimens, due to crush and edge artifacts. Thirdly only a small subset of 70 patients were studied due to limited time period of study. We use the ASCO/CAP IHC scoring criteria for HER2 expression used for gastroesophageal adenocarcinomas in interpreting the results. Maintaining a standard scoring system could omit the varied results published in literature. Also we limited our study to adenocarcinoma subtype only to omit any adultering effects from other histologic subtypes. Also all our patients were naive cases; none of them have received any systemic therapy which could alter the molecular phenotypes of the tumour clones. Despites these, we believe that future studies with larger sample size and the use of FISH for equivocal cases would be required to give a clearer picture.

CONCLUSION

Gastric and gastroesophageal junction adenocarcinomas is one of the aggressive diseases of mankind and often diagnosed at advanced stage. Surgery alone is not satisfactory and available cytotoxic drugs have limited efficacy. Establishing an effective targeted therapy required a consistently expressed molecule which undoubtly should play key role in the carcinogenesis. HER2 has established targeted drugs and role of HER2 in GEAs carcinogenesis is being more and more explored convincingly. The recently conducted ToGA trial has shown benefit of targeting HER2 receptors in metastatic and locally advanced GEAs. Our study revealed the incidence of HER2 overexpression in GEAs to be 12.9%. There was no statistically significant association of HER2 overexpression with age, gender, location of the tumour, specimen collection methods, lauren classification, stage or grades of the disease. Study with larger sample size are needed which would have statistical power to detect any such association if exists. We do hope our result will supplement the few datas available for Indian subjects and help generate the clinical relevance of HER2 status and the clinicopathologic characteristics.

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