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Association of Human Epidermal Growth Factor Receptor-2 (HER2) Over Expression with Subtype and Grade of Gastric Adenocarcinoma

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ABSTRACT

Background & Objective: Gastric cancer is associated with substantial morbidity and mortality worldwide. It is the fourth most commonly diagnosed cancer and the second most common cause of cancer related deaths globally. With increasing understanding of the molecular biology of HER2, and the availability of genomics and proteomics analysis, it has now been recognized that HER2 is implicated in other severe forms of cancers, notably gastric cancers. The objective of the study was to evaluate the frequency of over-expression of HER2 in patients of gastric adenocarcinoma.

Methodology: A retrospective cross sectional study was carried out in Head Office Chughtai Lab, Lahore from 1st July 2024 to 31st December 2024. A total of 65 diagnosed cases of gastric adenocarcinoma of both genders were included in the study. All autolyzed/poorly fixed specimens and metastatic gastric adenocarcinomas were excluded. The histological preparation was performed by classic method for inclusion in paraffin followed by hematoxylin and eosin staining. The immunohistochemical analysis was performed on serial sections using immune-enzymatic soluble complex method. The antibody used was HER2 polyclonal antibodies from DAKO. HER2 scoring was conducted as 0, +1, +2 and +3.

Results: Age range in this study was from 30 to 70 years with mean age of 56.492±11.91 years. Male gender was dominant (61.5%) as compare to females (38.5%). Over-expression of Her 2 was seen in 24.6% patients with gastric adenocarcinoma. Moreover, HER2 overexpression was found to be an independent prognostic factor in well to moderately differentiated adenocarcinomas with intestinal sub-type.

Conclusion: HER2 overexpression is a poor prognostic indicator in well to moderately differentiated, intestinal subtype-, early stage gastric adenocarcinomas. Therefore, IHC 3+ and 2+ cases should be further analyzed by FISH to assess HER2/neu gene status.

KEY WORDS: HER2, Adenocarcinoma, Gastric carcinoma, Immunohistochemistry

How to cite: *Fatima B, Afzal S, Manzoor F, Faisal M, Sadiq S, Chughtai A. Association of Human Epidermal Growth Factor Receptor-2 (HER2) Over Expression with Subtype and Grade of Gastric Adenocarcinoma. J Allam Iqbal Med Coll. 2025; 23(4): 110-113.*

INTRODUCTION

Gastric cancer is one of the most prevalent malignancies worldwide with increased morbidity and mortality. In advanced HER2-positive gastric adenocarcinoma, trastuzumab is added to first-line systemic chemotherapy based on HER2 expression, which is the primary biomarker that improves overall survival. [1,2]

Globally, gastric cancer ranks third in terms of cancer-related deaths and is the fifth most frequent type of cancer.[3] Since various therapeutic approaches that have been successful in treating breast cancer have not demonstrated clinical benefit in treating advanced gastric cancer, the unavoidable emergence of trastuzumab resistance continues to be a major issue.[4,5] Testing for human epidermal growth factor receptor 2 (HER2) has become standard procedure because it identifies gastric adenocarcinomas that respond well to targeted therapy. To choose suitable patients who will benefit from HER2-targeted treatment, accurate HER2 testing is essential. Between 4.4% and 53.4% of gastric malignancies have been documented to be HER2 positive, and these tumors are thought to exhibit more aggressive biologic behavior and tumor recurrence. In situ hybridization (ISH) for gene amplification and immunohistochemistry (IHC) for protein expression are the two primary HER2 testing techniques used in clinical practice.[6,7] Under trastuzumab maintenance monotherapy, a subgroup of patients with HER2-positive metastatic gastric and gastroesophageal junction tumors exhibits a sustained response.[8] Targeted cancer treatments are possible using biomarker-guided therapy. There are currently two HER 2-targeting alternatives for the treatment of metastatic HER2 positive gastric cancer: trastuzumab and trastuzumab- deruxtecan. However, because of variability and resistance mechanisms, these treatments rarely have long-lasting effect.[9,10] Different studies showed variable

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* Received : August 26, 2025
* Revised: October 28, 2025
* Corrected & Edited: November 20, 2025
* Accepted: December 10, 2025

expression of HER2 in different populations. There are only a few published studies among Pakistani population. The aim of this study is to examine the expression of HER2 in gastric carcinoma patients presenting to our setting so that they can get benefit from the drug trastuzumab which is effective in those showing positive IHC results for HER2.

METHODOLOGY

A retrospective cross-sectional study, using non-probability purposive sampling, was performed at Histopathology department Chughtai Institute of Pathology from 1st July 2024 to 31st December 2024 on 65 confirmed cases of gastric adenocarcinoma. All diagnosed cases of gastric adenocarcinoma on endoscopic or surgically resected specimens between ages of 25 to 85 years of both genders were included in the study. Blocks for review and immunohistochemistry from outside laboratories were also included. All poorly fixed specimens were rejected. Gastric adenocarcinomas at metastatic locations were not included. The histological preparation were performed by classic method for inclusion in paraffin followed by haematoxylin-eosin staining. The immunohistochemical analysis was performed on serial sections using immune-enzymatic soluble complex method. The antibody used was HER2 polyclonal antibodies from DAKO. HER2 scoring was conducted as per CAP guidelines for reporting HER2 expression in endoscopic biopsy and resection specimen. The cases with 3+ score of HER2 were taken as positive. Data was entered and analysed by using SPSS version 26. Mean and standard deviation were calculated for quantitative variables like age. Frequency and percentage were calculated for qualitative variables like gender, histological grade and subtypes of gastric adenocarcinoma. Effect modifiers like age, histological grade, gender and HER2 over-expression were controlled through stratification. Post stratification chi-square test was applied by taking P value equal to 0.05 as significant.

RESULTS

Age range in this study was from 30 to 70 years with mean age of 56.492±11.91 years. Male gender was dominant (61.5%) as compare to females (38.5%). Over-expression of HER2 was seen in 24.6% patients as shown in Table-I. Stratification of HER2 with respect to age, gender and histological grade are shown in Table-II, III and IV respectively.

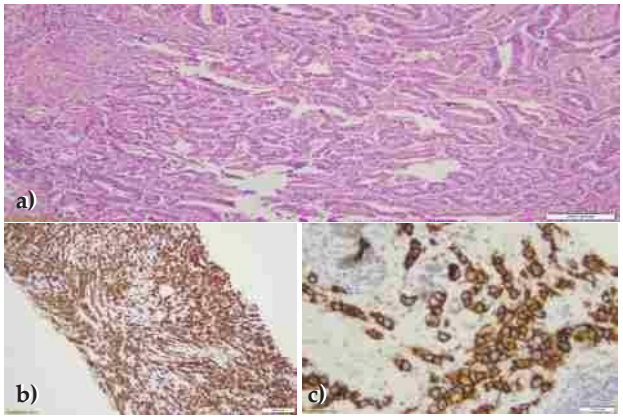

Fig 1: a) Photomicrograph (10X) of moderately differentiated gastric adenocarcinoma in a 45 years old patient. (b, c) (10X & 40X) The tumor was scored as +3 on IHC for HER2.

Table I: Frequency and Percentage of Patients According to Over-Expression of HER2 (n=65)

Over-expression of HER2	*No of Patients*	*%age*
Positive	16	24.6%
Negative	49	75.4%
Total	65	100%

Table II: Stratification of over-Expression of HER2 With Respect to Age.

Age (years)		*Over-expression of HER2*		*p-value*
		Yes	*No*	
1	30-50	5(21.7%)	18(78.3%)	0.690
2	51-70	11(26.2%)	31(73.8%)	
Total		16(24.6%)	49(75.4%)	

Table III: Stratification of Over-expression of HER2 with respect to gender

Gender		*Over-expression of HER2*		*p-value*
		Yes	*No*	
1	Male	12(30%)	28(70%)	0.202
2	Female	4(16%)	21(84%)	
Total		16(24.6%)	49(75.4%)	

Table IV: Stratification of Over-expression of HER2 with respect to Histological Grade

Histological Grade		*Over-expression of HER2*		*p-value*
		Yes	*No*	
1	Well Differentiated	2(11.8%)	15(88.2%)	0.186
2	Moderately Differentiated	9(36%)	16(64%)	
3	Poorly Differentiated	5(21.7%)	18(78.3%)	
Total		16(24.6%)	49(75.4%)	

DISCUSSION

In the present study, HER2 overexpression was observed in 16 (24.6%) of 65 gastric adenocarcinoma patients. Other researchers reported that the positive rates of HER2 over-expression ranged from 7% to 34% in gastric cancers.[11,12] The HER2 overexpression positivity rates varied in different researches, the reason of which may be the differences of IHC procedure, type of antibody used, sample size, and different scoring systems employed.[13] There was no relation-

ship observed between Her 2 overexpression, sex, age and histological grade (P>0.05). Another study by Li H et al had the similar results about the association of her 2 over expression with demographic features.[14] In landmark Trastuzumab for gastric cancer (ToGA) clinical trial, 341 (22%) of 1527 gastric cancers were HER2 positive by IHC[15]. HER2 positivity differed significantly by histologic subtype (intestinal-type 34%, diffuse-type 6%, mixed-type 20%) and according to the site of tumor [32% gastroesophageal junction (GEJ), and 18% gastric localization.[12,17] Aamir S et al also reported that HER2 overexpression/amplification was strongly associated with tumor histology (intestinal-type 22%, diffuse-type 2%, mixed-type 5%; P=0.005) and grade of differentiation.[16] However, there was no significant difference of HER2 positive rates between GEJ cancers and gastric localization cancers in our study. The reasons of higher HER2 overexpression in intestinal-type gastric cancer and GEJ cancer are complex and necessary to be investigated in depth in the future. Some studies revealed a significant correlation between HER2 overexpression and poorer survival. Li H et al reported that the 5 year survival rate of patients with HER2 amplification/overexpression was significantly poorer than that of patients with no amplification/overexpression.[14] Li F et al confirmed that tumors with HER2 amplification were associated with poor mean survival time and 5-year survival rates (21% vs. 63%; P <0.05), age, TNM stage, and HER2 amplification were found to be independently related to survival by multivariate analysis.[18] Literature also stated that HER2-positive patients have a poorer prognosis than those with HER2 negative.[12,14,17,18] In our research, intestinal-type, well to moderately differentiated cancers with HER2 overexpression/ amplification also exhibited short 5-year survival rates than HER2-negative cases, that too in the diffuse/mixed-type and advanced tumor stage. According to the investigations discussed above and in the present research, it is thought that HER2 overexpression may constitute an independent prognostic factor in intestinal type, well differentiated gastric cancer patients. HER2 located on cytomembrane was easy to bind with the human monoclonal antibody, so it was suitable for targeted therapy in tumors.[9,10] In ToGA phase III multicenter, an international clinical trial was conducted at 130 centers, the preliminary results show Trastuzumab combined with standard fluoropyrimidine plus cisplatin chemotherapy could significantly prolong survival versus the same chemotherapy alone in advanced HER2-positive gastric cancer.[15] Currently, the most common clinical techniques used to assess HER2 gene status are IHC for HER2 protein and FISH for HER2 gene amplification. IHC, as a preferred method is used widely in clinical laboratories. However, IHC may potentially be affected by tissue fixation, choice of primary antibodies, methods of antigen retrieval, and chromosome aneuploidy. In addition, the results of interpretation may vary among observers, because of using different scoring system for IHC. FISH is currently internationally recognized as the "gold standard" for the detection of Her 2 gene amplification with being fast, accurate, highly sensitive and specific. The concordance of HER2 protein expression and gene amplification in gastric cancers, however, has been controversial. Silva MR et al reported the concordance rate of 94% between HER2 overexpression by IHC and automated FISH in patients of gastric adenocarcinoma.[20] In the ToGA trial, the concordance between HER2 positivity by IHC and FISH was 87% and differences were largely due to FISH positive cases that were IHC 0/1+ 15. As per literature, HER2 overexpression in gastric cancers is mainly due to gene amplification. However, HER2 overexpression may occur by a number of different mechanisms, including transcriptional activation by other genes or post-transcriptional events.[21] Therefore, a standardization of IHC for HER2 in gastric cancer is clearly warranted. IHC (HercepTest TM) test scoring system, specific for gastric cancer established by Hofmann in 2008 was used in our study, and our results are also concordant with Hofmann et al.

CONCLUSION

HER2 overexpression may be used as an independent prognostic factor (poor) for well to moderately differentiated gastric adenocarcinomas with intestinal subtype. IHC 3+ and 2+ cases, therefore, should be further detected by FISH to assess HER2/neu gene status.

Limitations: Small sample size and lack of FISH for confirmation of all cases (positive, equivocal and negative on IHC) proved to be a limitation. Moreover, survival analysis by adequate patient follow up can also improve future outcomes.

Ethical Approval: This study was approved by the Institutional Review Board (IRB) of Chughtai Institute of Pathology Reference number: CIP/IRB/1321, dated: 23-1-2025.

Conflict of interest: None

Financial Disclosure: None

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BF, SA: Conceptualization & study design.

FM, MF: Data Collection and manuscript drafting.

SS: Data Analysis and critical review.

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