

ASSOCIATION OF HER2/NEU EXPRESSION WITH PATIENT PROGNOSIS IN PRIMARY GASTRIC ADENOCARCINOMA

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ABSTRACT

Background: Gastric carcinoma is the second leading cause of cancer-related deaths worldwide.

Objective: The purpose of this study was to investigate the relationship between HER2 overexpression and prognostic factors in Pakistani patients with gastric adenocarcinoma.

Methods: Over the course of a year, 200 patients with gastric cancer underwent immunohistochemical staining as part of a cross-sectional investigation. The ToGA trial criteria were used to assess HER2/neu expression through immunohistochemistry (IHC) in Gastric Adenocarcinoma, including +3 Staining, +2 Staining, +1 Staining, and 0 Staining, regardless of gender. Statistical evaluation was conducted using Chi-Square or Fisher Exact tests with GraphPad Prism 5 and SPSS version 19.

Results: In 70% of the cases examined, it was discovered that HER2 was overexpressed. This overexpression was found to be correlated with patients over the age of 40 at diagnosis, specific histological subtypes, and moderately differentiated grades of primary gastric adenocarcinoma. In 20% of cases, HER2 was also found to be overexpressed, but no connection was found between HER2 and patients' gender. It was observed that 37% of diffuse-type gastric adenocarcinomas and 75% of intestinal adenocarcinomas overexpressed HER2. Based on tumor differentiation, HER2 overexpression was observed in 90% and 80% of patients with well-differentiated (grade I) and moderately differentiated (grade II) adenocarcinomas, respectively. Additionally, 60% of patients with grade III poorly differentiated adenocarcinoma displayed HER2 overexpression.

Conclusion: To improve survival rates in cases of gastric adenocarcinoma, it is advised to examine all instances for HER2 overexpression closely. This allows for targeted molecular therapy, as is also seen in the overexpression of HER2 in diffuse-type gastric cancer. To improve survival rates, it's essential to closely examine all cases of gastric adenocarcinoma for HER2 overexpression and provide targeted molecular therapy. This is also seen in cases of diffuse-type gastric cancer where HER2 overexpression is present.

Key words: HER2, Gastric adenocarcinoma, diffuse type tumor and immunohistochemistry.

How to cite this article: Aamir S, Khan N, Riaz SK, Sheikh AK, Mushtaq M, Akbar A, Khan S. Association of HER2/NEU expression with patient prognosis in primary gastric adenocarcinoma. Pak Postgrad Med J 2023;34(3): 150-153

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DOI: <https://doi.org/10.51642/ppmj.v34i03.630>

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INTRODUCTION

With almost 0.9 million newly identified cases along with 0.7 million fatalities globally each year, gastric carcinoma is one of the world's most prevalent cancers. In Pakistan, where it is more common in those with low socioeconomic positions, it is also one of the main health problems. An increasing number of men over 40 are developing stomach cancer, which is on the rise in incidence.^{1,2} The two main subtypes are the intestinal and diffuse types of gastric adenocarcinoma. The remaining malignancies are classified as mixed subtypes or forms that are difficult to categorize. The form that is most common is the intestinal category, which is distinguished by noticeable glands and the cohesiveness of cells in tumors. The signet-ring or non-signet-ring morphology of the tumor cells that make up the diffuse subtype are diffusely invading the stomach

wall and are not well-differentiated and discohesive. This subtype has a higher prevalence in low-risk locations, is more common in females and younger patients, and is mostly linked to heritable genetic disorders.⁵ As a targeted molecular therapy for a number of malignancies, trastuzumab is presently being used more and more frequently. For this purpose, accurate determination of (Human Epidermal Growth Factor Receptor 2) HER2 status in such patients is a cornerstone in successful therapeutic achievement.⁴

HER2 is a member of the HER family of growth factor receptors and these receptors are located on the surface of different types of epithelial cells. Immunohistochemistry is recommended as the first test to be performed to assess the status of HER2 overexpression in gastric cancers.⁶⁻⁸ However, due to heterogeneous HER2 expression and often incomplete membranous staining, the immunohistochemical scoring system is different from that of the breast cancer.⁹

In developing countries like Pakistan, very few studies have been carried out regarding the expression HER2 in gastric carcinoma. This study will significantly be beneficial for patients receiving targeted therapy even if they present in the advanced stage of the disease.^{10,11}

METHODS

This was a cross-sectional study conducted at Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad. Biopsies and resection specimens of patients (15 to 90 years of age) with histopathological diagnosis of gastric adenocarcinomas were included in the study irrespective of gender for the period of 1 year. Ethical approval was obtained from the institutional committee prior to the study. Demographic data along with relevant clinical and radiological details were noted. Patients with non-epithelial malignancies and stomach metastatic carcinoma were excluded from the study.

The HER2 immunohistochemistry was carried out using the HercepTest™ kit from Dako, Agilent Technologies, Inc., CA, USA, in accordance with the manufacturer's instructions. For Gastric Adenocarcinoma, HER2 overexpression was assessed using the criteria for scoring HER2/neu expression by immunohistochemistry (IHC) applied in the ToGA trial. A positive result is noted for +3 Staining if more than 10% of the neoplastic cells in the surgical resection specimen or at least a single group of cancer cells consisting of more than 5 cells in the biopsy sample show membranous staining. The staining should be of moderate to strong intensity in the basal and lateral parts of the tumor cell membranes. For +2 Staining, more than 10% of the neoplastic cells in the surgical resection specimen or at least a single group of cancer cells comprising more than 5 cells in the biopsy sample showed membranous staining. The staining should be of weak to moderate intensity in the basal and lateral parts of the tumor cell membranes. This staining intensity is considered equivocal. A negative result is obtained for +1 Staining when there is barely perceptible staining of tumor cell membranes shown by more than 10% of the neoplastic cells in surgical resection specimens or at least a single group of cancer cells comprising more than 5 cells in the biopsy sample. When even less than 10% of the neoplastic cells in surgical resection specimens or at least a single group of cancer cells comprising of more than 5 cells in the biopsy sample shows no membranous staining, 0 Staining is also considered a negative result. For further analysis, cases with a staining intensity of +3 and confirmed FISH +2 were considered as HER2 positive or overexpression. On the other hand, cases with staining intensities of +2, +1, and 0 were categorized as HER2 negative.

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 19 and GraphPad Prism version 5. Qualitative variables such as gender, type, grade of the tumor, and overexpression of HER2 in adenocarcinoma were calculated for frequency and percentage. For quantitative variables like the age of the patient, the Mean Standard Deviation (MSD) was calculated. To compare the variables, the Chi-square test or Fisher Exact test was performed, with a p-value less than 0.05 considered significant for the analyzed data.

RESULTS

The study included two hundred cases in which only 20 were resection specimens and 180 were gastric biopsies. We observed that gastric adenocarcinoma primarily affected individuals after 40 years of age having 79% of cases in the older group (Table 1). The patients had an age range from 21 to 80 years with the mean age being 52 years and a standard deviation of 12.8. The majority of them were male patients with male to female ratio being 1.8:1.

In our study, 69% of patients harbored Lauren's intestinal type adenocarcinoma and 31% were of diffuse type. These adenocarcinomas were graded according to the guidelines of the College of American Pathologists and it was

observed that only 17% of cases were diagnosed as grade 1 adenocarcinoma. Grade 2 cases comprised 26% whereas grade 3 consisted of 57% of cases (Suppl. Figure 1).

We observed that 70% of cases were recorded as positive showing an HER2 immunoreactivity score of 3+. The HER2 overexpression was observed in both types of gastric adenocarcinoma. Almost 75% intestinal-type cases (figure 1) and 60% diffuse-type cases (figure 2) showed staining intensity of +3 (table 1).

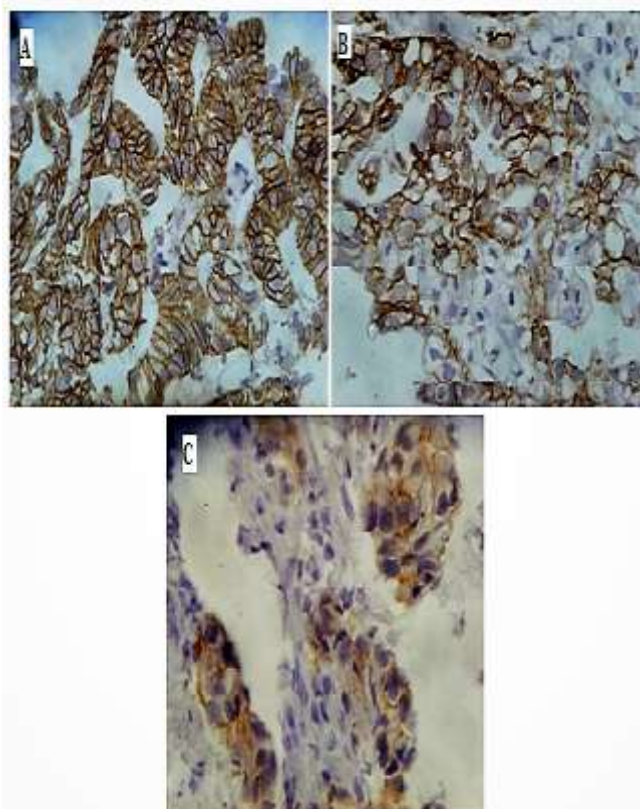


Figure 1: Microscopic picture of intestinal type gastric adenocarcinoma showing A). HER2 staining intensity of +3 (x100) B). HER2 staining intensity of +2. (x400) C). HER2 staining intensity of +1 . (x400)

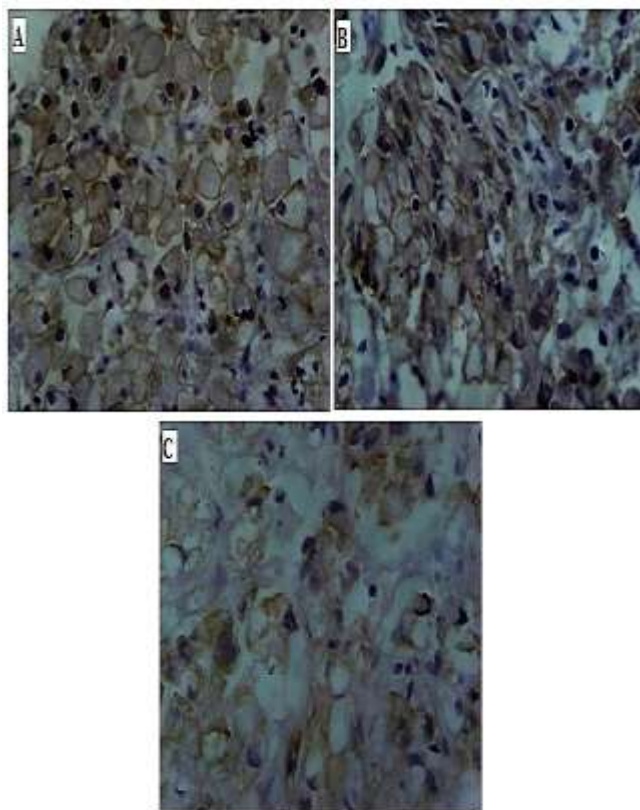


Figure 2: Microscopic picture of diffuse type gastric adenocarcinoma showing A). HER2 staining intensity of +3 B). HER2 staining intensity of +2 C). HER2 staining intensity of +1 at 400x.

Overexpression of HER2 was significantly observed in 80% of patients diagnosed with gastric cancer above the age of 40 years. HER2 expression was higher in 73% male patients and 64% female patients. (Table 1, figure 3).

We found that HER2 overexpression was manifested by grade 1 and grade 2 tumors, comprising of 90 and 80% respectively. However, 60% patients with grade 3 tumors also demonstrated significant HER2 overexpression (table 1, figure 3).

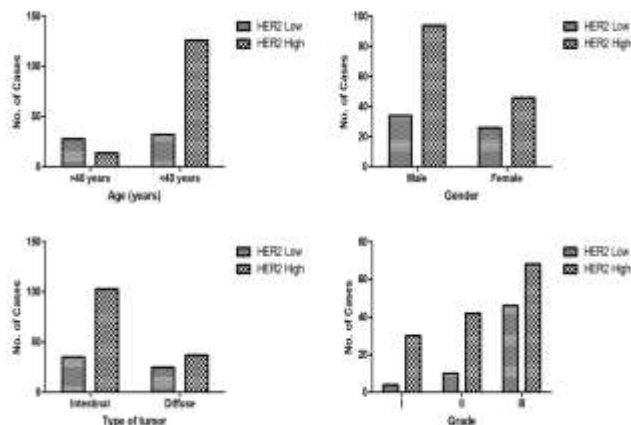


Figure 3: Association of HER-2 expression with different prognostic factors in Gastric carcinoma patients.

Table 1: Clinical and histopathological features of Gastric carcinoma cases and association with expression of HER-2.

Parameters	Number of cases (%)	HER2 Low (%)	HER2 High (%)	p-value
Age at presentation				
≤40 years	42 (21)	28(66)	14(34)	<0.0001 *
>40 years	158 (79)	32 (20)	126 (80)	
Gender				
Male	128 (64)	34 (27)	94 (73)	0.2*
Female	72 (36)	26 (36)	46 (64)	
Histological type				
Intestinal	138 (69)	35 (25)	103 (75)	0.04
Diffuse	62 (31)	25 (40)	37 (60)	
Grade				
I	34 (17)	4 (10)	30 (90)	0.0009
II	52 (26)	10 (20)	42 (80)	
III	114 (57)	46 (40)	68 (60)	

*Fisher Exact Test; Bold figures show

DISCUSSION

In our study, two hundred cases with histopathological diagnosis of gastric adenocarcinoma were evaluated for HER2 expression by immunohistochemistry. Various studies have quoted the frequency of HER2 overexpression in a range of 4.4 % to 53.4% of gastric carcinoma cases with a mean of 17.9%.¹³⁻¹⁶ In this study, 70% cases were recorded as positive for HER2 overexpression. Contrastingly, studies conducted on Korean and Indian population documented HER2 overexpression in 27% cases.¹⁴⁻¹⁶ Numerous factors may contribute towards this variation in data e.g., use of different antibodies, differences in size of sample, varying methods of evaluation, and use of different scoring systems for evaluation of HER2 overexpression.¹⁷

HER2 overexpression was most frequently found, in the present study, in the old age group with 80% of the HER2 positive patients in the 41-80yrs age range. Similar findings were reported in South Asian patient.^{16, 18} Moreover, 73% of male patients and 64% of female patients recorded as HER2 positive cases. Similarly, HER2-positive tumors were more frequently found in older patients and male gender previously.^{7, 19}

Regarding type of gastric carcinoma, it was found that 75% HER2 positive cases had presented as intestinal-type adenocarcinoma whereas 60% cases as diffuse type

adenocarcinoma. In a study cohort of 99 gastric carcinoma patients, 12% cases showing HER2 overexpression were documented. It was observed that 19% cases of intestinal-type adenocarcinoma and 6% of diffuse-type adenocarcinoma had shown HER2 overexpression.^{7, 20} These results are comparable to the present study. On the contrary, 22% of intestinal-type adenocarcinoma and 0 % of diffuse type adenocarcinoma exhibited HER2 overexpression thus, showing the association of HER2 overexpression with intestinal-type gastric adenocarcinoma in another study.¹⁸ In a recent study, 34% of intestinal-type adenocarcinoma and 15% of diffuse type were observed to have HER2 overexpression.²⁰

The frequency of HER2 overexpression in different grades of gastric adenocarcinoma was also analyzed. It was found that staining intensity of +3 was mostly manifested by grade I (well-differentiated) and grade II (moderately differentiated) tumors. However, 60 % of patients with grade III had also shown the same intensity of HER2 overexpression in our study. A similar trend of association has been observed in the South Asian gastric cancer patients.¹⁸

It is concluded from this study that overexpression of HER2 receptor protein is observed in both intestinal and diffuse-type gastric adenocarcinoma. A significant association of HER2 overexpression was found with prognostic factors like age, tumor type, and grade of the patients. Immunohistochemistry was found to be an easy and reliable technique to determine the HER2 status of gastric adenocarcinoma. It is, therefore, suggested that HER2 overexpression should be determined by immunohistochemistry in every patient presenting with gastric carcinoma so that targeted molecular therapy can be offered to these patients for overall better survival. Also, its overexpression in low-grade adenocarcinoma reassures such patients if the targeted therapy is given at the initial stages. It is the need of time to enhance the insight by investigating further genetic subtypes and using a larger sample size for authentication of this study. As there is therapeutic importance of HER2 diagnosis therefore further studies will lead to better prognosis of patients in the future.

Conclusion: To improve survival rates in cases of gastric adenocarcinoma, it is advised to examine all instances for HER2 overexpression closely. This allows for targeted molecular therapy, as is also seen in the overexpression of HER2 in diffuse-type gastric cancer. To improve survival rates, it's essential to closely examine all cases of gastric adenocarcinoma for HER2 overexpression and provide targeted molecular therapy. This is also seen in cases of diffuse-type gastric cancer where HER2 overexpression is present.

Ethical Approval: Submitted

Conflict of Interest: Authors declare no conflict of interest.

Funding Source: None

REFERENCES

- Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. *Cancer Manag Res.* 2018; 10:239-248.
- Yousaf A, Tasneem N, Mustafa A, Fatima R, Nabia N, Khan RA, Abdulbasit H, et al. Gastric cancer associated risk factors and prevalence in Pakistan. *ASEAN J Sci Eng.* 2021;1(2):738
- Rosai and Ackerman's Surgical Pathology. 11th ed. New Delhi: Elsevier Inc; 2018. p. 528-567.

4. Saletti P, Zaniboni A. Second-line therapy in advanced upper gastrointestinal cancers: current status and new prospects. *J Gastro Oncol*. 2018 Apr;9(2):377.
5. Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R. Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies. *Intern J Mol Sci*. 2020 4;21(11):4012.
6. Matsuoka T, Yashiro M. Biomarkers of gastric cancer: Current topics and future perspective. *World J Gastroenterol*. 2018 7;24(26):2818.
7. Patne SCU, Abhilash VB, Dixit VK, Katiyar R, Kumar S, Singh GP. Immunohistochemical Expression of Human Epidermal Growth Factor Receptor 2 (HER2) and p53 in Gastric Adenocarcinoma: A Pilot study from Northern India. *J Clin Diagn Res*. 2017;11(5):EC43-EC45.
8. Grassini D, Cascardi E, Sarotto I, Annaratone L, Sapino A, Berrino E, Marchiò C. Unusual patterns of HER2 expression in breast cancer: Insights and perspectives. *Pathobiology*. 2022 3;89(5):278-296.
9. Zhao D, Klempner SJ, Chao J. Progress and challenges in HER2-positive gastroesophageal adenocarcinoma. *J Hematol Oncol*. 2019 17;12(1):50.
10. Kelly CM, Janjigian YY. The genomics and therapeutics of HER2-positive gastric cancer—from trastuzumab and beyond. *J Gastrointest Oncol*. 2016 ;7(5):750.
11. Abrahao-Machado LF, Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. *World J Gastroenterol*. 2016 5;22(19):4619.
12. Wu X, Chen S, Lin L, Liu J, Wang Y, Li Y, et al. A Single Domain-Based Anti HER2 Antibody Has Potent Antitumor Activities. *Transl Oncol*. 2018;11(2):366-373.
13. Ibrahim M, Gilbert K. Management of gastric cancer in Indian population. *Transl Gastroenterol Hepatol*. 2017; 2:64.
14. Pye H, Butt MA, Funnell L, Reinert HW, Puccio I, Rehman Khan SU, et al. Using antibody directed phototherapy to target oesophageal adenocarcinoma with heterogeneous HER2 expression. *Oncotarget*. 2018;9(33):22945-22959.
15. Han S, Park S, An J, Yang JY, Chung JW, Kim YJ, Kim KO, et al. HER2 as a potential biomarker of lymph node metastasis in undifferentiated early gastric cancer. *Scientific Reports*. 2020 24;10(1):5270.
16. Nadaf AS, Rani H, Dinesh US. Immuno- Histochemical Assessment of HER2NEU Expression in Gastric Adenocarcinoma in North Karnataka, India. *Asian Pacific J Cancer Prevent*. 2018;19(5):1381-1385.
17. Phan DAT, Nguyen VT, Hua TNH, Ngo QD, Doan TPT, Nguyen ST, et al. HER2 Status and Its Heterogeneity in Gastric Carcinoma of Vietnamese Patient. *J Patho Transl Med*. 2017;51(4):396-402.
18. Shabbir A, Qureshi MA, Khalid AB, Mirza T, Shaikh A, Hasan SM. Gastric adenocarcinoma expressing human epidermal growth factor receptor in South Asian population. *Saudi J Gastroent Assoc*. 2018; 24(5):289-293.
19. Battaglin F, Naseem M, Puccini A, Lenz HJ. Molecular biomarkers in gastro-esophageal cancer: recent developments, current trends and future directions. *Cancer Cell Int*. 2018 11; 18:99. doi: 10.1186/s12935-018-0594-z. e Collection 2018.
20. Sakaguchi M, Shimoike N, Akagawa S, Kanaya S. Strategy for treatment of stage IV human epidermal growth factor 2 positive gastric cancer: a case report. *J Med Case Rep*. 2019 22; 1. 3(1):42.