ORIGINAL RESEARCH

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Clinicopathological Factors in Relation to HER2 Status in Metastatic Gastric Cancer: A Retrospective Observational Study

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ABSTRACT Objective: Data available on the rate of human epidermal growth factor receptor 2 (HER2) positivity and clinicopathological parameters related to it are heterogeneous. Hence, it is pertinent to investigate these parameters in different populations. This study aims to determine the frequency of HER2 positivity and clinicopathological factors associated with it in metastatic gastric cancer patients in a Turkish population. **Material and Methods:** This study included 552 patients with metastatic gastric cancer from 5 oncology centers. HER2 status, age, gender, smoking and alcohol history, body mass index, basal carcino embryonic antigen (CEA) level, basal cancer antigen 19-9 (CA 19-9) level, tumor localization, de-novo metastatic cancer, Lauren classification, signet-ring cell component, venous and neural invasion, and histological grade data were collected retrospectively. HER2 positivity was defined as an immunohistochemistry (IHC) score of 3+ or an IHC score of 2+ and in situ hybridization positive. Univariable and multivariable logistic regression analyses were used to detect clinicopathological factors associated with HER2 status. **Results:** A total of 100 patients (18.1%) were HER2-positive. Alcohol consumption, basal CEA level, basal CA 19-9 level, and signet-ring cell component were found to be statistically significant in univariable analysis. Alcohol use, basal CEA level, and having signet-ring cell component were statistically significant in the multivariable analysis. Odds ratios of alcohol use, high basal CEA and having signet-ring component were 2.35 (95% confidence interval (CI): 1.27-4.36, p=0.006), 1.99 (95% CI: 1.19-3.36, p=0.009), and 0.39 (95% CI: 0.22-0.71, p=0.002) respectively. **Conclusion:** HER2 positivity was detected in 18.1% of metastatic gastric cancer patients in the Turkish population. Alcohol use and basal CEA level were positively, and the signet-ring cell component was negatively correlated with HER2 positivity.

Keywords: Gastric cancer; HER2; clinicopathological factors; ISH

Gastric cancer [including adenocarcinoma of the stomach and gastroesophageal junction (GEJ)] is the fifth most common cancer diagnosed and the third most common cancer-causing death. Most of the patients are diagnosed in the advanced stage, and the 5-year survival rate is less than 10% at this stage.[1] The standard treatment of advanced-stage gastric cancer is chemotherapy.[2]

Advancement in the field of molecular pathways in different cancer types led to the development and use of targeted therapies like trastuzumab, a monoclonal antibody that binds to human epidermal growth factor receptor 2 (HER2). Adding it to the chemotherapy in the treatment of HER2-positive, advanced-stage gastric adenocarcinoma patients improved survival in the Phase 3 Trastuzumab for

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Gastric Cancer (ToGA) trial. Subgroup analysis in the ToGA trial revealed that survival benefit is more prominent in groups of immunohistochemistry (IHC) score 2+ and fluorescence in situ hybridization (FISH) positive or IHC score 3+ for HER2.[3,4] The results of the trial changed the clinical practice of treatment of HER2-positive advanced stage gastric cancer patients.

HER2 positivity frequency in gastric cancer is between 7% and 38%, as reported in different populations.[5,6] The relationship between clinicopathological parameters and HER2 status has been investigated earlier. Tumor localization, intestinal type, male gender, grade, serum carcino embryonic antigen (CEA) level, and the metastatic site were found to be associated with HER2 positivity in these studies.[7,8] However, the results are conflicting; hence, it is important to investigate the HER2-related clinicopathological factors in different independent populations.

This study aims to investigate HER2 positivity frequency and clinicopathological factors associated with HER2 status in metastatic gastric cancer patients in a Turkish population.

MATERIAL AND METHODS

STUDY DESIGN AND PATIENTS

In this retrospective multicenter study, 552 histopathologically diagnosed metastatic gastric cancer patients (>18 years old, female and male) who have been followed between the years of 2017 and 2021 from 5 different cancer centers have been included. The data of the patients were collected from the hospital databases of these centers. The pathological examination was performed, and HER2 status was determined in each center independently. Formalin-fixed, paraffin-embedded primary or metastatic tumor specimen was used for IHC and in situ hybridization (ISH) evaluations. Automated IHC, FISH, and silver ISH analyses were carried out in the Ventana BenchMark Ventana (Roche, USA) platform in every center by using the Ventana 4B5/Thermo SP3 antibody, the PathVysion (Abbott, USA) HER-2 FISH probe, and Ventana HER2 Dual ISH probe, respectively. HER2 positivity was defined as either an IHC score of 3+ or an IHC score of 2+ (equivocal) and ISH positive [HER2/centromere of chromosome 17≥2.0]. Age, gender, smoking and alcohol history, body mass index, basal CEA level, basal cancer antigen 19-9 (CA 19-9) level, tumor localization, being metastatic at the diagnosis, Lauren classification (intestinal, diffuse, mixed, and non-classified), signet-ring cell component, venous and neural invasion, and histological grade data were collected and studied as clinicopathological factors. The general characteristics of the whole population, the HER2 status of the patients, and the association between HER2 status and clinicopathological factors were determined.

Approval of the Ethical Committee was obtained from the Ankara University Faculty of Medicine Ethics Committee (01.09.2020, İ7-460-20) in compliance with the Helsinki Declaration.

STATISTICAL ANALYSIS

All parameters were used as categorical variables and presented as numbers and percentages. Univariable and multivariable logistic regression analyses were used to determine the relationship between clinicopathological factors and HER2 status. Only variables that were important in the univariable analysis were included in the multivariable analyses. All p values were calculated using a 2-tailed significance test (p=0.05). IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA) was used to conduct the statistical analyses.

RESULTS

GENERAL CHARACTERISTICS

We included 552 patients with metastatic gastric cancer in this study. The general characteristics of the study population are given in Table 1. 35.7% of the patients were above 65 years of age, and 31.5% were female. 51.2% of the patients were smokers, and 16.9% of the patients had a history of alcohol intake. 46.2% of the patients were either overweight or obese. High CEA and CA 19-9 were detected in 42.1% and 40.8% of the patients at the diagnosis. The tumor was localized at the GEJ in 21.4% of patients, while 53.6% of the patients had de-novo metastatic

TABLE 1: General characteristics of the study population.

	n=552
Age n (%)	
≤65	355 (64.3)
>65	197 (35.7)
Gender n (%)	
Female	174 (31.5)
Male	378 (68.5)
Smoking n (%)	
Yes	283 (51.2)
No	269 (48.8)
Alcohol n (%)	
Yes	93 (16.9)
No	459 (83.1)
BMI n (%)	
<18.5	33 (6)
18.5-24.9	264 (47.8)
25-29.9	200 (36.3)
≥30	55 (9.9)
Basal CEA n (%)	
Normal	320 (57.9)
High	232 (42.1)
Basal CA 19-9 n (%)	
Normal	327 (59.2)
High	225 (40.8)
Localization n (%)	
GEJ	118 (21.4)
Stomach	434 (78.6)
Lauren classification n (%)	
Intestinal	197 (35.6)
Diffuse	212 (38.4)
Mixed	29 (5.4)
Non-classified	114 (20.6)
Signet-ring cell component n (%)	
Yes	203 (36.8)
No	349 (63.2)
Venous invasion n (%)	
Yes	408 (73.9)
No	144 (26.1)
Neural invasion n (%)	
Yes	297 (53.8)
No	255 (46.2)
Histological grade n (%)	
Good	15 (2.7)
Intermediate	162 (29.3)
Bad	375 (68)
De-novo metastatic n (%)	
Yes	296 (53.6)
No	256 (46.4)

BMI: Body mass index; CEA: Carcino embryonic antigen; CA 19-9: Cancer antigen 19-9; GEJ: Gastroesophageal junction.

cancer. The number of patients with an IHC score of 2+ and an IHC score of 3+ was 81 and 69, respectively. As per the Turkish Health Ministry regulations, the ISH test was not done in patients with IHC score of 1+. Overall, 100 (18.1%) patients were HER2-positive according to the above mentioned definition (Table 2).

CLINICOPATHOLOGICAL VARIABLES AND HER2 STATUS

To find clinicopathological parameters linked to HER2 positivity, univariable and multivariable logistic regression analyses were performed (Table 3). Alcohol use, basal CEA, and basal CA 19-9 levels, having signet-ring cell component were found statistically significant in univariable analysis. Alcohol use, basal CEA level, and having signet-ring cell component were statistically significant in the multivariable analysis. Odds ratios of alcohol use, high basal CEA and having signet-ring cell component were 2.35 (95% confidence interval (CI): 1.27-4.36, p=0.006), 1.99 (95% CI: 1.19-3.36, p=0.009), and 0.39 (95% CI: 0.22-0.71, p=0.002) respectively. Alcohol use and high basal CEA level were positively correlated, whereas having a signet-ring cell component was negatively correlated with HER2 positivity.

DISCUSSION

HER2 positivity was 18.1% in our study. Alcohol use, basal CEA level, and signet-ring cell component were found to be associated with HER2 positivity. HER2 positivity was more prevalent in patients with high basal CEA levels and alcohol use, while less common in patients with signet-ring cell component. Age, gender, smoking history, body mass index, basal CA 19-9 level, tumor localization, being metastatic at the diagnosis, Lauren classification, venous and neural invasion, and grade were not associated with HER2 status.

TABLE 2: IHC and ISH results of the patients.

	ISH negative n	ISH positive n	Total n
IHC score 3+	20	61	**81***
IHC score 2+	49	**19***	68
IHC score 1+	-	-	403

-Only IHC score 2+ and IHC score 3+ patients have ISH test; *HER2-positive: IHC score 2+ plus ISH positive or IHC score 3+, Total HER2-positive patient number: 100 (18.1%); IHC: Immunohistochemistry; ISH: In situ hybridization.

TABLE 3: Univariable and multivariable logistic regression analysis for HER2 status.

Variable	HER2-positive (n=100)	HER2-negative (n=452)	Univariable p OR (95% CI)	Multivariable p OR (95% CI)
Age n (%)				
≤65	56 (56)	299 (66)	0.056	
>65	44 (44)	153 (34)	1.53 (0.98-2.38)	
Gender n (%)				
Female	27 (27)	147 (33)	0.28	
Male	73 (73)	305 (67)	1.3 (0.8-2.1)	
Smoking n (%)				
Yes	48 (48)	235 (52)	0.55	
No	52 (52)	217 (48)	0.87 (0.55-1.37)	
Alcohol n (%)				
Yes	24 (24)	68 (15)	**0.04**	**0.006**
No	76 (76)	384 (85)	**1.75 (1.01-3.04)**	**2.35 (1.27-4.36)**
BMI n (%)				
<18.5	7 (7)	27 (6)		
18.5-24.9	45 (45)	217 (48)	0.80	
25-29.9	37 (37)	163 (36)	1.04 (0.74-1.46)	
≥30	11 (11)	45 (10)		
Basal CEA n (%)				
Normal	44 (44)	276 (61)	**0.002**	**0.009**
High	56 (56)	176 (39)	**2 (1.28-3.12)**	**1.99 (1.19-3.36)**
Basal CA 19-9 n (%)				
Normal	47 (47)	280 (62)	**0.007**	0.58
High	53 (53)	172 (38)	**1.84 (1.18-2.87)**	1.64(0.98-2.73)
Localization n (%)				
GEJ	26 (26)	92 (20)	0.21	
Stomach	74 (74)	360 (80)	0.72 (0.44-1.2)	
Lauren classification n (%)				
Intestinal	44 (44)	154 (34)		
Diffuse	29 (29)	185 (41)	0.28	
Mixed	10 (10)	18 (4)	0.89 (0.72-1.1)	
Non-classified	17 (17)	95 (21)		
Signet-ring cell component n (%)				
Yes	21 (21)	181 (40)	**0.001**	**0.002**
No	79 (79)	271 (60)	**0.4 (0.23-0.68)**	**0.39 (0.22-0.71)**
Venous invasion n (%)				
Yes	79 (79)	330 (73)	0.35	
No	21 (21)	122 (27)	1.42 (0.66-3.04)	
Neural invasion n (%)				
Yes	49 (49)	249 (55)	0.46	
No	51 (51)	203 (45)	0.78 (0.41-1.49)	
Histological grade n (%)				
Good	3 (3)	14 (3)		
Intermediate	35 (35)	127 (28)	0.32	
Bad	62 (62)	311 (69)	0.79 (0.50-1.25)	
De-novo metastatic n (%)				
Yes	54 (54)	242 (54)	0.93	
No	46 (46)	210 (46)	1.01 (0.66-1.57)	

HER2: Human epidermal growth factor receptor 2; OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; CEA: Carcino embryonic antigen; CA 19-9: Cancer antigen 19-9; GEJ: Gastroesophageal junction.

In the study of Park et al. from South Korea, 11.7% of the 813 gastric cancer patients studied were HER2-positive. High CEA level, well-differentiation, pulmonary and distance lymph node metastasis were found to be predictors of HER2 positivity.[9] Our study has found a similar relationship with the CEA level. In another study with 228 locally advanced and metastatic gastric cancer patients, HER2 positivity was 24.6%. Male gender and diffuse-type were associated with HER2 positivity.[10] In a study from Japan, HER2 positivity was 21.2%, and intestinal type, absence of peritoneal metastasis, and presence of liver metastasis were related to the HER2 positivity.[11] HER2 positivity was 9.8%, and intestinal type, well differentiation, and GEJ localization were associated with the HER2 positivity in the study of Shan et al.[12] The ToGA trial also investigated the tumor localization and histological type in relation to HER2 status. HER2 positivity was more common in patients with intestinal-type and GEJ localization.[7] Another study with 197 patients did not show any relationship between tumor localization and HER2 positivity.[13] Though HER2 positivity was higher in patients with intestinal-type and GEJ localization in our study, it was not statistically significant. The discrepancy in the correlation of parameters and conflicting results among studies may partly be attributed to the differences in the geographical and genetic backgrounds of the populations studied. The differences in HER2 status determination and scoring system may also contribute to the disparity in results.

A meta-analysis in 2017 included 15 studies to evaluate clinicopathological factors associated with HER2 status. Male gender (odds ratio (OR): 1.42; 95% CI: 1.23-1.64), good/intermediate differentiation (OR: 2.76; 95% CI: 1.72-4.45), and intestinal-type (OR: 0.31; 95% CI: 0.25-0.38) were found to be associated with HER2 positivity.[8] Another meta-analysis of 41 studies revealed male gender (OR: 1.48; 95% CI: 1.34-1.65), proximal tumor (OR: 1.25; 95% CI: 1.07-1.47), intestinal-type (OR: 3.37; 95% CI: 2.54-4.47), lymph node metastasis (OR: 1.26; 95% CI: 1.14-1.41), well-differentiated cancer (OR: 1.79; 95% CI: 1.15-2.76) and distant metastasis (OR: 1.91; 95% CI: 1.08-3.38) were related to the HER2 positivity.[14] Although it was not statistically significant, HER2 positivity was greater in the male gender in our study. There was no correlation between histological grade and Lauren classification. HER2 positivity was found to be related to cardia tumor in several studies.[14] Another study revealed that alcohol use increases the risk of cardia tumors while it does not increase non-cardia tumors.[15] In our study, alcohol use was associated with HER2 positivity. Further studies are imperative to understand the role of alcohol consumption and its molecular mechanism in HER2 positivity.

There are several limitations of our study. Because the study was multicenter and pathological specimens were evaluated by each center independently, there may be variation in the reporting of HER2 status and other pathological features. Eating habits, tumor localization in the stomach (fundus, antrum, corpus, pylori, and curvature), metastasis sites, and other possible parameters were not included in the study.

To the best of our knowledge, this is the first comprehensive study that investigates HER2 status and clinicopathological factors in gastric cancer in Turkey. The relationship between high CEA level and HER2 status may pave the way for conducting prospective studies in the future. Because HER2 is a therapeutic target, it is crucial to evaluate HER2 status at the diagnosis in patients with signet ring cell component.

CONCLUSION

HER2 positivity was detected 18.1% in metastatic gastric cancer patients in a Turkish population. Alcohol use and basal CEA level were positively, and the signet-ring cell component was negatively correlated with HER2 positivity.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Mustafa Gürbüz, İzzet Doğan, Erman Akkuş, Filiz Çay Şenler;* ***Design:*** *Mustafa Gürbüz, İzzet Doğan, Erman Akkuş, Filiz Çay Şenler;* ***Control/Supervision:*** *Filiz Çay Şenler;* ***Data Collection and/or Processing:*** *Mustafa Gürbüz, İzzet Doğan, Erman Akkuş, İbrahim Karadağ, Serdar Karakaya, Cihan Erol, Ramazan Acar, Mert Karaoğlan, Elif Berna Köksoy, Berna Savaş, Mehmet Ali Nahit Şendur, Didem Taştekin, Nuri Karadurmuş, Berna Öksüzoğlu, Filiz Çay Şenler;* ***Analysis and/or Interpretation:*** *Mustafa Gürbüz, İzzet Doğan, Erman Akkuş, Filiz Çay Şenler;* ***Literature Review:*** *Mustafa Gürbüz, İzzet Doğan, Erman Akkuş, Filiz Çay Şenler;* ***Writing the Article:*** *Mustafa Gürbüz, İzzet Doğan, Erman Akkuş, Filiz Çay Şenler;* ***Critical Review:*** *Filiz Çay Şenler.*

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