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ORIGINAL ARTICLE

Evaluation of Her2 Positivity in Gastric Cancer Using Two Different Methods: A Prospective Study

Mide Kanserinde Her2 Pozitifliğinin Farkli İki Yöntemle Değerlendirilmesi: Prospektif Bir Çalışma

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ABSTRACT

Aim: Gastric cancer, a common type of gastrointestinal (GI) system malignancy, has an incidence associated with various factors. These include ethnic background, dietary habits, socioeconomic status, lifestyle, geographic region, and the prevalence of Helicobacter pylori. As with all malignancies, the pathogenesis of gastric cancer involves various genetic factors, one of which is the human epidermal growth factor receptor-2 (EGFR-2) (Her2/neu or c-erbB2) gene. The objective of this study is to compare the amplification levels of the Her2/neu gene in gastric cancer samples obtained from our patients using Real-Time polymerase chain reaction (RT-PCR) with the overexpression of Her2 protein, assessed through immunohistochemistry (IHC) on the same tissue samples. Additionally, the study aims to investigate the relationship between Her2 positivity and prognostic factors such as age, stage, gender, tumor localization, and histological type.
Method: This study was conducted prospectively on newly diagnosed 50 gastric cancer patients. Her2 gene expression at the mRNA level was assessed using RT-PCR on both tumor and normal fresh gastric tissue samples from all 50 patients. Additionally, Her2 protein levels were evaluated through IHC on paraffin blocks from these same 50 patients, and the results were compared with each other.
Results: In the evaluation of IHC, Her2 overexpression was observed in five patients (10%), and Her2 amplification was detected in five patients (10%) using RT-PCR. However, it was notable that there was no correlation between these two methods, and the result was statistically insignificant ($p > 0.05$). Additionally, no statistically significant difference was observed between Her2 gene expression in tumor tissues and prognostic factors ($p > 0.05$).
Conclusion: Her2 protein overexpression and Her2 gene amplification may contribute to potential tumor development. As no correlation was found between these two methods, further studies are needed to evaluate false negativity, investigate patient survival in terms of its contribution to prognosis, and validate our hypothesis.

Keywords: Gastric cancer, Her2 (c-erbB2), immunohistochemistry, Real-Time polymerase chain reaction

ÖZ

Amaç: Gastrointestinal sistem malignitelerinin sık görülen bir tipi mide kanserinin insidansı çeşitli faktörlerle ilişkilendirilmiştir. Etnik köken, beslenme şekli, sosyoekonomik durum, yaşam tarzı, coğrafi bölge koşulları ve H. pylori prevalansı ile değişkenlik göstermektedir. Tüm malignitelerdeki gibi gastrik kanserin patogenezinde çeşitli genetik faktörler yer almaktadır ve human epidermal büyüme faktörü reseptörü 2 (Her2/neu ya da c-erb B2) geni de bunlardan biridir. Gastrik kanser hastalarımızdan alınan örneklerde Her2/neu geninin Real-Time polimeraz zincir reaksiyonu (RT-PCR) ile amplifikasyon düzeyini, yine aynı dokudan alınmış ve IHC ile çalışılan Her2 protein overekspresyonu ile karşılaştırmaktır. Pozitiflik saptandığı durumda yaş, evre, cinsiyet, tümoral lokalizasyon ve histolojik tip gibi prognostik faktörlerle ilişkisini araştırmaktır.
Yöntem: Bu çalışma, prospektif olarak yeni tanı alan 50 mide kanseri hastası üzerinde yapılmıştır. Elli hastanın hem tümöral ve hem de normal taze mide dokusundan RT-PCR yöntemiyle mRNA düzeyinde Her2 gen ekspresyonu bakılmıştır. Yine bu elli hastanın IHC değerlendirme için parafin bloklar üzerinden Her2 protein seviyesi çalışılmıştır ve bu sonuçlar birbirleriyle karşılaştırılmıştır.
Bulgular: IHC ile yapılan değerlendirmede beş hastamızda (% 10) Her2 overekspresyonu ve Real-Time PCR ile beş hastamızda (% 10) amplifikasyon saptandı. Ancak bu iki yöntem arasında korelasyon bulunmadığı dikkatimizi çekti ve istatistiksel olarak anlamsızdı ($p > 0.05$). Ayrıca tümör dokularındaki Her2gen ekspresyonları ile prognostik faktörler arasında istatistiksel olarak anlamlı bir fark gözlenmedi ($p > 0.05$).
Sonuç: HER2 protein overekspresyonu ve Her2 gen amplifikasyonu potansiyel tümör gelişimine katkıda bulunabilir. Bu iki yöntem arasında korelasyonun olmaması sonucunda; yanlış negatifliğin değerlendirilmesi ve prognoza katkısı açısından hasta sağkalımlarının araştırılması gerekmektedir, bu hipotezimizin doğrulanması için daha ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Mide kanseri, Her2 (c-erb B2), immunohistokimya, Real-Time PCR

Introduction

Gastric cancer is a common malignancy of the gastrointestinal (GI) tract (1-3). It is one of the leading causes of cancer-related mortality worldwide (3,4). Its incidence varies depending on both environmental and genetic factors, such as ethnicity, geographic conditions, dietary habits, socioeconomic status, lifestyle, and the prevalence of Helicobacter pylori (1,3,4).

Among the genetic factors contributing to gastric cancer, the inactivation of adenomatous Polyposis coli (APC) and tumor protein 53 (tP53), the most frequently inactivated genes among tumor suppressor genes, as

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well as mutations, amplifications, or overexpressions in the proto-oncogenes rat sarcoma (RAS), cellular myelocytomatosis (C-myc), and human epidermal growth factor receptor-2 (EGFR-2) (Her2), a member of the EGFR family, have been identified (5,6).

The Her2 protein (also known as c-erbB2/neu) is a transmembrane tyrosine kinase receptor encoded by the Her2/neu gene located on the long arm of chromosome 17. It is primarily responsible for regulating various cellular events, including differentiation, proliferation, apoptosis, and cell survival. Her2 proteins contribute to tumor cell biology by enhancing cell proliferation through amplification and protein overexpression resulting from mutations in tumor tissues, disrupting apoptosis, and participating in processes such as adhesion and migration (1,2,7). These findings suggest that Her2 is an oncogene.

Studies have shown that the Her2 gene plays a role in many types of cancer. Her2 amplification and/or overexpression has been detected in approximately 34% of invasive breast cancer cases and other cancer types such as colon, esophageal, gastric, bladder, endometrial, and lung cancers, as well as head and neck tumors (2,8).

Several studies have demonstrated the importance of Her2 in the pathogenesis, prognosis, and novel treatments of gastric cancers. Additionally, several studies have investigated anti-Her2 agents in response to the need for new therapeutic approaches in aggressive gastric cancers (8-12).

In light of this information, the objective of this study is to immunohistochemically assess Her2 receptor levels in the tumor tissue of newly diagnosed gastric cancer patients who have not received any prior treatment, to detect Her2/neu gene expression at the mRNA level using the polymerase chain reaction (PCR) method in both normal and tumor gastric tissues, to compare these levels, and to examine their effects on prognosis. Given that Her2 overexpression and increased Her2 activity are poor prognostic factors, any increase in Her2 expression could be used as a prognostic biomarker in diagnosis and as a therapeutic target in treatment. In summary, we aim to contribute to the development of new gastric cancer treatment strategies using anti-Her2 agents, thereby increasing patients' life expectancy.

Material And Method

Study Design

The protocol for this prospective study was approved by the Gaziantep University Medical Ethics Committee before the commencement of the study (Approval No. 115, Approval Date: March 13th, 2012). The study sample consisted of 50 patients who visited the Gastroenterology Polyclinic at our hospital or were referred from an external center for endoscopy, underwent endoscopy at the Endoscopy Unit, were diagnosed with gastric adenocarcinoma in the pathology department, and volunteered for the study. Informed consent forms were obtained from each patient included in the study.

If a peptic ulcer or tumor formation was detected during the endoscopy procedures of the patients selected for this study, biopsies were taken from both normal and tumor gastric tissues for Her2/neu gene analysis, after obtaining the relevant consent from the patients, in addition to the biopsy taken for pathological evaluation.

Clinical Diagnosis and Data Collection

After obtaining the relevant informed consent from patients with suspected gastric cancer, biopsies were taken during the endoscopy procedures performed at the Endoscopy Unit. Patients' data, including age, gender, complaints at admission, and whether they had received treatment for known stomach diseases, were collected from their medical histories. Localization was determined by endoscopy. All patients underwent a physical examination. Patients' demographic characteristics and family histories were recorded. Pathological evaluation, along with imaging methods, including abdominal and thoracic CT scans, were performed to determine the cancer stages and to detect the histological type, differentiation, invasion, and lymph node involvement. The pathological evaluation of patients with early gastric cancer or resectable advanced gastric cancer, following surgical procedures, was conducted at the Pathology Laboratory of the Faculty of Medicine. Tissue samples from patients diagnosed with gastric cancer were tested for Her2 positivity by immunohistochemical (IHC) staining.

Her2/neu gene expression was investigated in both normal and tumor gastric tissue samples taken from patients whose gastric cancer was diagnosed based on pathological findings, using the Real-Time PCR (RT-PCR) method after RNA isolation in the Medical Genetics Laboratory.

Immunohistochemical Assessment

The diagnosis of gastric adenocarcinoma in the patients was made in the Department of Pathology by IHC analysis using antibodies developed against Her2. Formalin-fixed paraffin blocks were deparaffinized with xylene solutions after being heated at 60°C in an oven. IHC staining was performed via the immunoperoxidase method, followed by chromogenic staining. Subsequently, the preparations were examined under light microscopy using primary rabbit monoclonal HER2 antibody (1:100), and those showing diffuse field staining were evaluated. The percentage and intensity of the staining were scored as '-', indicating no staining; '1+', indicating light staining; '2+', indicating moderate staining; and '3+', indicating strong staining (Table 1).

Table 1. IHC Scoring System in Gastric Cancer

Membrane Staining Pattern	Assessment Results	Additional Evaluation
No staining reactivity	Negative	Not necessary
Light or indistinct membranous staining	Negative	Not necessary
Moderate basolateral or lateral membranous staining	Doubtful	ISH Correlation Recommended
Strong basolateral or lateral membranous staining	Positive	Not necessary

IHC: Immunohistochemistry, ISH: In situ hybridization

Samples scored as '-' were considered negative, and those scored as '3+' were considered positive. On the other hand, samples scored as '1+' and '2+' were re-evaluated with fluorescence in situ hybridization (FISH) (13).

RNA Isolation

The tubes containing tumor and normal tissue samples obtained by endoscopy were stored in a liquid nitrogen tank and then sent to the Medical Genetics Laboratory. The samples were removed from liquid nitrogen and divided into 25 mg pieces. RNAs were extracted using the QIAamp RNA Blood Mini Kit following the manufacturer's recommended method. After centrifugation, the supernatant was removed. The samples were placed on a filter tube and centrifuged. Next, 90 µl of DNase Incubation Buffer and 10 µl of DNase I solution were added to the filter tube and incubated at room temperature for 15 minutes. 500 µl of Wash Buffer I was added to the filter tube and centrifuged. Subsequently, 500 µl of Wash Buffer II was added to the filter tube and centrifuged. Then, 300 µl of Wash Buffer II was added and centrifuged. After centrifugation at 8,000xg for 1 minute, the RNAs were removed from the filter tube and stored at -85 °C.

cDNA Synthesis

Copy DNAs (cDNAs) were synthesized using the Ipsogen Reverse Transcription-Dx Kit. PCR conditions were adjusted, and the PCR mixture was prepared as specified in the kit. Necessary amounts of RNAs were obtained by measuring RNAs using a nanodrop. They were prepared for a total of 100 samples; 50 were tumor tissue samples, and the other 50 were normal tissue samples. PCR was performed using the AB Applied Biosystems Veriti 96-Well Thermal Cycler PCR device.

Real-time polymerase chain reaction (RT-PCR)

Detection of Her2 gene expression by RT-PCR: The Her2 gene region was studied using the Qiagen primer set.

RT-PCR Determination of ACTB expression: Actin Beta (ACTB) was used as the housekeeping gene, and the following mixture was prepared for a total of 100 samples to compare Her2 and ACTB.

Statistical Analysis

Statistical analyses of the collected data were conducted using SPSS 15.0 (Data Analysis Software System, Version 15.0, 2006). The Kolmogorov-Smirnov test was used to analyze the normal distribution characteristics of the continuous variables. In the comparison of two independent groups, the student's t-test was used for normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables. The relationship between categorical variables was analyzed using Pearson's chi-square test. The descriptive statistics obtained from the collected data were expressed as frequencies (n), percentages (%), and mean ± standard deviation values. Probability (p) values of < 0.05 were considered statistically significant. Pearson's correlation analysis was used to investigate the correlations between different parameters.

Results

The sample for this prospective study consisted of 50 patients who presented to the Department of Gastroenterology and were diagnosed with gastric cancer based on endoscopic procedures. Of these patients, 34 (68%) were male and 16 (32%) were female, yielding a male-to-female ratio of 2.1:1. The mean age of the sample was 60.22 ± 14.7 years, with a range from 29 to 88 years.

Among the 50 patients, 28 (56%) had previously sought medical attention for stomach-related complaints

and had a history of gastritis, peptic ulcer disease, or gastroesophageal reflux disease. Additionally, 19 (38%) patients, including 16 males and 3 females, were using proton pump inhibitors (PPIs) as part of their treatment regimen. Furthermore, 39 (78%) patients, including 4 with a history of GI bleeding, were taking acetylsalicylic acid (ASA) and/or nonsteroidal anti-inflammatory drugs (NSAIDs) (Table 2).

Table 2. Distribution of Patients' Socio-Demographic Characteristics by Gender

	Male (n=34)	Female (n=16)
Mean Age (years)	61.38±11.47	57.75±20.17
Patients with comorbidities	68%	32%
Patients with known stomach disease	20	8
Patients with a history of PPI use	16	3
Patients with a history of ASA or NSAID use	29	10
Patients with a history of GI bleeding	4	0
Patients with a history of familial cancer	19	9
Patients with a history of familial gastric cancer	0	3

A total of 28 patients (56%) had a familial history of cancer. Among them, three patients (6%) had a family history specifically of gastric cancer, and all of these individuals were female (Table 2).

Among the 50 patients, 28 (56%) had comorbidities, all of whom were on additional medications. Within this group, 4 patients (8%) had only hypertension, 5 (10%) had heart disease, 10 (20%) had diabetes, and 3 (6%) had cancer. Additionally, 39 patients (78%) were using ASA and/or NSAIDs, and 19 (38%) were using PPIs. The study identified 33 active smokers (66%), while 7 patients (14%) reported alcohol use. Notably, all patients who consumed alcohol were also active smokers, representing 14% of the sample. Among the 50 patients diagnosed with gastric adenocarcinoma, 17 (34%) had the signet ring cell type, 3 (6%) had a mixed type, and the remaining 30 (60%) were classified with other types of adenocarcinoma.

Based on the staging of gastric cancers among the patients, 3 patients (6%) were classified as stage 1, 7 (14%) as stage 2, 8 (16%) as stage 3, and 32 (64%) as stage 4. A total of 44 patients (88%) exhibited lymph node metastases, while 6 patients (12%) showed no lymph node involvement.

IHC assessments conducted after the pathological evaluation of gastric cancer diagnoses revealed that 41 patients had negative Her2 staining, 3 (6%) exhibited 1+ staining, 1 patient (2%) exhibited 2+ staining, and 5 (10%) exhibited 3+ Her2 staining. Patients with 1+ and 2+ Her2 staining underwent additional evaluation using silver in situ hybridization (SISH), which confirmed negative Her2 status. In contrast, patients with 3+ Her2 staining were deemed positive for Her2 without requiring further SISH testing. Overall, only 5 patients (10%) were found to have Her2 overexpression based on 3+ IHC staining.

Analysis of Her2 overexpression by gender revealed that 4 patients (80%) with Her2 positivity were male, while 1 patient (20%) was female. There was no significant difference in age between patients with Her2 positivity and those with Her2 negativity. Similarly, no statistically significant correlation was observed between tumor localization and Her2 positivity (p=0.375). None of the 17 patients with signet ring cell adenocarcinoma or the 3 patients with mixed-type adenocarcinoma exhibited Her2 positivity. Her2 negativity in patients with signet ring cell adenocarcinoma was considered borderline significant (p=0.056). Additionally, no significant correlation was identified between cancer stage, metastasis, and Her2 positivity (p=0.845) (Table 3).

Table 3. Distribution of Her2 Overexpression by Patient Subgroups

Her2 Overexpression (IHC)			***p*-value**
	Patients with Her2-Negativity	Patients with Her2-Positivity	
Localization:			0.375
Upper zone	7 (15.6%)	1 (20%)	
Trunk	10 (22.3%)	2 (40%)	
Pyloric antrum	12 (26.7%)	1 (20%)	
Multiple zones	16 (35.5%)	1 (20%)	
Histological Type:			0.056
Signet ring	17 (37.8%)	0 (0%)	
Mixed	3 (6.7%)	0 (0%)	
Other	25 (55.6%)	5 (100%)	
Cancer Stages: I	3 (6.7%)	0 (0%)	0.845
II	6 (13.3%)	1 (20%)	
III	7 (15.6%)	1 (20%)	
IV	29 (64.4%)	3 (60%)	

IHC: Immunohistochemistry

To evaluate Her2 gene amplification in our patients, the housekeeping gene ACTB was used to calculate Her2 levels in tumoral and normal tissue samples. Patients with a ratio above 1.25 (n=5) were classified as having Her2 amplification, while those with a ratio below 0.75 (n=3) were classified as having decreased Her2 expression.

All 5 patients with Her2 gene amplification were male. Among three patients with decreased Her2 expression, two were female, and one was male. Regarding tumor localization, three patients (60%) with Her2 gene amplification had multi-zone involvement, one (20%) had involvement in the pyloric antrum and one (20%) in the gastric trunk. No significant relationship was found between tumor localization and gene amplification (p=0.459). Similarly, there was no significant association between histological type and gene amplification (p=0.290). Three of five patients (60%) with Her2 gene amplification were cases of stage IV cancer. However, no significant correlation was observed between cancer stage and gene amplification (p=0.382) (Table 4).

Table 4. Distribution of Her2 Gene Amplification by the Subgroups of Patients

	Her2 Gene Amplification (PCR) T/N tissue ratio			*p*-value
	Amplified	Normal	Decreased	
Localization:				
Upper zone	0 (0%)	8 (19.0%)	0 (0%)	0.459
Trunk	1 (20%)	11 (26.2%)	0 (0%)	
Pyloric antrum	1 (20%)	10 (23.8%)	2 (66.7%)	
Multiple zones	3 (60%)	13 (31.0%)	1 (33.3%)	
Histological Type:				
Signet ring	2 (40%)	14 (33.3%)	1 (33.3%)	0.290
Mixed	1 (20%)	1 (2.4%)	1 (33.3%)	
Other	2 (40%)	27 (64.3%)	1 (33.3%)	
Cancer Stages:				
I	0 (0%)	3 (7.1%)	0 (0%)	0.382
II	1 (20%)	6 (14.3%)	0 (0%)	
III	1 (20%)	4 (9.5%)	3 (100%)	
IV	3 (60%)	29 (69.0%)	0 (0%)	

PCR: Polymerase chain reaction

Furthermore, no significant correlation was identified between Her2 gene amplification and Her2 protein overexpression as determined by IHC (p=0.397) (Table 5).

Table 5. Comparison of Her2 Overexpression and Gene Amplification

	Her2 Gene Amplification (PCR) T/N tissue ratio			
IHC assessment	**Amplified**	**Normal**	**Decreased**	***p*-value**
Negative	5 (100%)	37 (88.1%)	3 (100%)	0.397
Positive	0 (0%)	5 (11.9%)	0 (0%)	
Total	5 (100%)	42 (100%)	3 (100%)	

IHC: Immunohistochemistry, PCR: Polymerase chain reaction

Discussion

Surgery is the cornerstone of gastric cancer treatment and is supplemented with chemotherapy as neoadjuvant or adjuvant therapy. Given the relatively low overall survival rates associated with standard treatment regimens for gastric cancer, targeted therapies have gained significant attention. In this context, various studies have been conducted targeting the Her2 gene, known as an oncogene, which has become a focal point of research, and Her2-positivity was determined as a poor prognostic factor for breast cancer. Consequently, trastuzumab, an anti-Her2 agent, became one of the standard agents in the treatment of breast cancer (9, 14).

The methods used to assess Her2 gene amplification and protein overexpression were primarily developed based on studies investigating Her2 positivity in breast cancer. FISH and IHC are the two most commonly employed methods for primary assessment. Among these two options, IHC is the most widely used due to its affordability and speed. However, as a quality control-dependent method, IHC can produce subjective and relatively variable results. In contrast, FISH is more expensive but offers greater reliability. The reported correlation between FISH and IHC results ranges from 73% to 98% (9, 15-17). Consistent with the literature, this study utilized the IHC method to assess protein overexpression and the PCR method to evaluate gene amplification.

Her2 overexpression in gastric cancer was first studied by Sakai et al. in 1986 using the IHC method (18). Subsequent studies have reported Her2 positivity rates in gastric cancer ranging from 8.5% to 32%, depending on the methods used. It has been concluded that Her2 positivity is a poor prognostic factor in gastric cancer patients due to its association with lymph node involvement, an aggressive disease course, and shorter survival (2, 19-21). However, other studies argue that Her2 positivity is not linked to prognosis or lymph node involvement. They attribute findings of Her2 positivity as a poor prognostic factor to methodological differences, including variations in techniques used to assess Her2 overexpression, the choice of IHC antibody, sample size, histopathological subtypes, degree of differentiation, and stage of disease (22, 23).

The wide range of Her2 positivity rates (8.5% to 32%) reported in gastric cancer studies may be attributed to factors such as tumor histology, patient population, age group, disease stage, and, most importantly, the method used to determine Her2 overexpression. Even among studies employing the same method,

variations in methodological details, such as the specific antibody used for IHC, stand out as significant contributors to these differences (24, 25).

Notably, there are key distinctions in evaluating Her2 overexpression with the IHC method in gastric cancer compared to breast cancer. Firstly, membranous staining reactivity tends to be incomplete in gastric cancer. Secondly, tumoral heterogeneity is observed at a higher frequency in gastric cancer than in breast cancer (13, 26, 27).

In a meta-analysis by Wang et al. (28) investigating potential correlations between Her2 overexpression and various parameters, including age, gender, localization, stage, and histological type, Her2 overexpression was found to be positively associated with intestinal type, advanced stage, and disease spread. In contrast, in this study, the correlation between Her2 overexpression and histological type was only borderline significant ($p = 0.056$).

In another study examining Her2 positivity using IHC and SISH, 7 of 14 IHC 2+ patients were evaluated as SISH positive, while all 8 IHC 3+ patients were found to be SISH positive. This study concluded that as the depth of invasion increases, Her2 negativity also increases ($p=0.03$) (29). In our study, the depth of invasion was not evaluated.

Kimura et al. (22) reported no significant relationship between Her2 positivity and stage, lymph node involvement, or invasion in gastric cancer, concluding that Her2 positivity lacks prognostic value. However, the Her2 positivity rate was determined to be 24% using the IHC method.

In the study conducted by Baykara et al. (30), IHC evaluation was performed, and FISH analysis was applied specifically to the low IHC-positive group. A positivity rate of 15% was observed in this patient cohort. Furthermore, it was highlighted that Her2 positivity in early-stage cases is linked to a worse prognosis.

In another study, Her2 positivity in gastric cancer was reported at 8%. In this study, all 7 patients with IHC 2+ and one of the 12 patients with IHC 3+ were found to be FISH negative (31).

In a review by Chua et al. (32), it was stated that IHC is the most commonly used method for determining Her2 positivity, followed by ISH. The study reported an average Her2 positivity rate of 18%, based on data from several studies, and noted that Her2-positive patients tend to have shorter lifespans compared to Her2-negative patients. Her2 positivity was also identified as a poor prognostic factor. However, it was concluded that Her2 positivity is not correlated with parameters such as age, gender, localization, tumor size, invasion, or stage. In comparison, this study determined that five of the 50 patients exhibited Her2 positivity based on IHC findings. Further analysis of four patients with low Her2 staining using FISH revealed that these patients were, in fact, negative for Her2. In this study, Her2 positivity was not associated with age, gender, tumor localization, or stage ($p>0.05$). Based on the histopathological evaluation of tissue biopsies and the presence of a signet ring cell component, patients were categorized into three gastric adenocarcinoma groups: signet ring cell type, mixed type, and other types. The IHC assessment revealed no Her2-positive cases in the signet ring cell or mixed-type groups. The patients found to be Her2-positive all had other types of gastric adenocarcinoma. The difference in Her2 positivity between the "other types" group and the signet ring cell and mixed type groups was borderline significant ($p = 0.056$).

In the study by Gürbüz et al. (33), Her2 positivity was detected at a rate of 18.1% in IHC evaluation. Additionally, it was noted that the presence of a signet-ring cell component was negatively correlated with Her2 positivity.

In a study comparing Her2 positivity using IHC and FISH, samples from 10 patients included only gastric biopsies, 10 patients had only gastric resection specimens, and 20 patients had both biopsy and gastric resection specimens (a total of 40 patients). Her2 positivity was confirmed with both methods in nine patients (22.5%), and heterogeneity was observed in 5 patients. This heterogeneity was validated using both FISH and IHC in both biopsy and surgical resection specimens (34). As previously emphasized, heterogeneity is more common in gastric cancer compared to breast cancer.

In a study investigating the relationship between Her2 positivity and atypical gastric tumors, Giuffré et al. (35) determined that Her2 positivity, as evaluated by IHC, is associated with high-grade and advanced-stage gastric cancer. As a result, they concluded that Her2 overexpression is a poor prognostic factor linked to low survival rates and high mortality.

PCR analysis revealed gene amplification in 5 out of the 50 patients included in this study. No significant correlation was found between positive gene

amplification and factors such as age, localization, histological type, or stage ($p > 0.05$). Additionally, there was no significant correlation between the results obtained through PCR and IHC methods ($p > 0.05$). Notably, gene amplification was not detected in patients identified as having Her2 overexpression by the IHC method, and conversely, Her2 overexpression was not observed in patients with gene amplification determined by the PCR method.

Risio et al. (36) emphasized that the timing of IHC analysis significantly impacts the results. They reported that as the time between the preparation of paraffin blocks and the IHC analysis increases, the rate of Her2 negativity also rises. In this study, the IHC assessment of samples collected from 50 patients over two years was conducted collectively at the end of the study. Consequently, a higher Her2 positivity rate was observed in the samples from patients with more recent diagnoses.

In a study evaluating Her2 positivity exclusively in tumoral tissues using three different methods, Kim et al. (25) reported that Her2-positive cases were associated with moderate-to-well-differentiated tumors. They also found no relationship between Her2 positivity and factors such as age, stage, or gender. Patients assessed with FISH were further analyzed using PCR, revealing a 97.6% concordance between the two methods. Notably, four patients who were determined to have Her2-negative staining via the IHC method were found to exhibit gene amplification through PCR. Additionally, in one patient with 1+ Her2-positive staining by IHC, PCR detected high amplification, while FISH yielded a negative result.

In a study comparing the results of gene amplification assessment using the RT-PCR method with heterozygosity and IHC results, Königshoff et al. (37) stated that the RT-PCR method has limited use due to difficulties in sample stabilization. They noted that if stabilization is not achieved, RNA degradation will increase, leading to a decrease in the reliability of RT-PCR. Additionally, they mentioned that gene amplification is a tissue-limited phenomenon, highlighting the importance of heterogeneity. They emphasized a correlation between transcriptional or post-translational activation in patients with positive IHC staining and the absence of gene amplification detected by PCR. They attributed the finding that patients identified as having gene amplification by the PCR method exhibited negative IHC staining to the lack of the promoter region in the area where amplification was detected. Furthermore, they indicated that if amplification had occurred recently, the protein overexpression step might not have been initiated yet. In other words, they stressed that there is a certain time lag between amplification and overexpression. They also stated that the probability of obtaining negative results with the IHC method increases as time elapses before the paraffin blocks are analyzed.

Another important concern that should be noted in patients with positive IHC staining and no gene amplification is the content of the sample obtained by biopsy. As Dabbs et al. (38) stated, even if the tissue is directly taken from the tumoral area, it may contain a small or insufficient amount of invasive tumor tissue. Additionally, since the tissue biopsied from the tumoral region includes peripheral tissue elements such as adipose tissue, fibrous tissue, necrotic tissue, and connective tissue, as well as immune system components like reactive cells, lymphocytes, and macrophages, tumor mRNA can be diluted, resulting in a false-negative PCR result. Furthermore, the gastric intratumoral area has a heterogeneous structure. Thus, the likelihood of false-negative results increases if the biopsied portion coincides with a region exhibiting low or no gene amplification of Her2. Conversely, biopsy samples taken from patients with positive gene amplification may show IHC staining ranging from 1+ and 3+ due to this heterogeneity. These false-negative results contribute to a low correlation between IHC and PCR results in patients with positive gene amplification. Therefore, it is widely believed that at least two different methods, such as IHC and FISH, should be used before treatment to confirm the results obtained with PCR.

In two separate studies conducted by Lemoine (39) and Kameda (40), a low correlation was found between gene amplification and protein overexpression rates. They suggested that gene amplification is not the primary mechanism in patients who exhibit high overexpression despite negative amplification results. They noted that various genes can mediate overexpression, which may occur through increased transcriptional activation or alterations in post-transcriptional processes. Conversely, more recent studies have reported higher correlations between gene amplification and protein overexpression rates assessed by different methods.

O'Malley et al. (41) investigated the correlation between Her2 evaluation results obtained using three different IHC techniques and those obtained through

the PCR method. They found that the results from the three IHC techniques correlated with the PCR results in 82%, 89%, and 80% of the patients, respectively. Additionally, IHC staining results were negative in 8 patients who were identified as having positive gene amplification by the PCR method. This finding suggests that even the use of three different high-sensitivity antibody detection kits was insufficient to detect low protein levels. Conversely, the PCR results for 11 patients who exhibited positive IHC staining across all three antibody types were negative. The authors attributed the negative gene amplification results from the PCR method in these 11 patients' tumor tissue samples to the heterogeneity of the samples.

The HER2 positivity of gastric cancer was evaluated in the Trastuzumab for Gastric Cancer (ToGA) study conducted by Bang et al. (42). As a result, trastuzumab was incorporated into treatment protocols for gastric cancer patients as a first-line therapy. In the study, an 87% concordance rate was observed between the results obtained using the IHC and FISH methods. Targeting Her2 positivity as a therapeutic approach significantly improved survival time. One of the study's most important findings was that anti-Her2 agents are ineffective in patients with positive gene amplification unless there is overexpression of the Her2 protein.

In this study, Her2 positivity was detected in 10% of gastric carcinoma patients using the IHC method, and gene amplification was also identified in 10% of gastric carcinoma patients using the PCR method. These rates are consistent with those reported in the literature. However, despite reports in the literature indicating a 60% to 95% correlation between the results obtained by the two methods, no significant correlation was found between them in this study. The use of the ISH method may be more effective in addressing the inconsistency between these methods and reducing false-negative results. Considering that the rate of negative IHC results increases as the time between preparation and analysis of paraffin blocks lengthens, it is essential to analyze paraffin blocks immediately after preparation.

In this study, biopsies were taken from both tumoral and normal tissues, and pathological evaluation reports were available for the tumoral tissues. However, tissues deemed normal were assessed macroscopically rather than histopathologically. Therefore, it should be noted that tissues considered normal may contain tumor cells. This could affect the amplification rate in patients with normal or low amplification levels, potentially leading to false-negative results.

Given the significance of heterogeneity in gastric cancer, the biopsy specimen used for IHC analysis must be adequate. An insufficient biopsy specimen can obscure heterogeneity and increase the likelihood of false-negative results. Additionally, care must be taken to prevent the tissue intended for gene amplification studies from becoming diluted.

In conclusion, while targeted treatments such as trastuzumab are effective in gastric cancer patients with Her2 overexpression, we believe that evaluation methods for Her2 require further validation through clinical studies. Therefore, future researches, including analyses of other mutations and polymorphisms, are needed to uncover potential relationships.

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Ethics Committee Approval

Gaziantep University Medical Ethics Committee, Approval No: 115, Date: March 13th, 2012.

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Conflict of Interest

The authors have no conflicts to disclose concerning the authorship and/ or publication of this article.

Author contributions

AIHS, AS, and MA contributed to the study design. AIHS, IS, SO, and BC collected the data. AIHS, IS, and SO analyzed the data and ensured the accuracy of the data analysis. AIHS, IS, MA, and SO interpreted the data and drafted the manuscript. AIHS, IK, and MA critically revised the manuscript for important content. All authors read and approved the final manuscript.

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