



HER 2 Expression in Gastric Cancer

Mide Kanseriinde HER 2 Ekspresyonu

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Özet

Amaç: Mide kanseri birçok ülkede insidansı azalmakla birlikte dünya genelinde sık görülen kanserlerden biri olmaya devam etmektedir. Mide kanseri, birçok genetik ve epigenetik değişiklikleri içeren biyolojik olarak heterojen bir hastalıktır. Hastalığın bu heterojenitesine rağmen aynı evredeki hastalar benzer tedavileri almakta idi. HER 2 ekspresyonunun immünohistokimyasal olarak veya florasan in situ hibridizasyon ile gösterildiği metastatik mide kanserli hastalarda transtuzumab'ın sağkalım avantajı göstermesiyle bu durum değişmektedir. Bu nedenle mide kanserli hastalarda HER 2 ekspresyon oranını bilmek önemlidir. Bu çalışmada immünohistokimya ile mide kanserli hastalarda HER 2 ekspresyon oranı araştırılmıştır. **Gereç ve Yöntem:** Antalya Eğitim ve Araştırma Hastane'sinde 2008-2011 yılları arasında tanı konulan 50 mide kanserli hasta çalışmaya alındı. **Bulgular:** Mide kanserli 50 hastanın doku örneklerinde HER 2 ekspresyonu, 25'inde (% 50) 0 olarak, 11'inde (%22.7) 1, 7'sinde (%14) 2, 7'sinde (%14) 3 olarak skorlandı. Pozitif oranı yaklaşık % 14 (7/50) idi. Her 2 ekspresyon durumu TNM evresi, lenf nodu durumu, uzak metastaz ve yaş ile korele değildi. (p:0.344, p:0.315, p:0.181, p:0.96) Her 2 ekspresyon durumu cinsiyet ile korele idi. (p:0.041) HER-2 pozitif olan hastaların tümü erkekti. **Tartışma:** Çalışmamızda sadece IHC yöntemi kullanılmış ve 2 pozitif olan hastalar HER 2 ekspresyonu negatif olarak kabul edilmiştir. Meme kanserinden 2 pozitif hastaların bir kısmında FISH yöntemi ile HER 2 ekspresyonunun olduğu bilinmektedir. Bu nedenle HER 2 ekspresyon oranı sapadığımız değerden farklı olabilir.

Anahtar Kelimeler

Mide Kanseri; HER 2; İmmünohistokimya

Abstract

Aim: Even though gastric cancer incidence decline in many countries, it is still among the mostly witnessed cancers in the world. Gastric cancer is a biologically heterogeneous disease with many genetic and epigenetic variations. Despite this heterogeneity of the illness, patients in same stages received similar treatments. This changes as transtuzumab shows survival advantages in patients with metastatic gastric cancer. Therefore it is important to know the rate of HER 2 expression in patients with gastric cancer. In this study, we examined the rate of HER 2 expression in patients with gastric cancer by immunohistochemical method. **Material and Method:** A total of 50 patients with gastric adenocarcinoma who underwent diagnosed at Antalya Education and Research Hospital from 2008 to 2011 were enrolled in this study. **Results:** HER 2 expression of the 50 gastric carcinoma in tissue samples, 25 (50%) were scored as 0, 11 (22%) as 1, 7 (14%) as 2, and 7 (14%) as 3. The positive rate was approximately 14% (7/50). The HER-2 status was not correlated with the TNM stage, lymph node status, distant metastasis and age (p:0.344, p:0.315, p:0.181, p:0.96). The HER-2 status was correlated with sex (p:0.041). All of the HER-2 positive patients were male. **Discussion:** In our study only IHC method was performed and patients who had a score of 2+ were considered to have negative HER 2 expression. It is known that some of the patients with breast cancer with a score of 2+ established HER 2 expression by FISH method. Therefore, we think that HER 2 expression ratio may differ from the values we have obtained.

Keywords

Gastric Cancer; HER 2; Immunohistochemistry

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Introduction

Even though gastric cancer incidens decline in many countries, it is still among the mostly witnessed cancers in the world [1]. The disease demonstrates a fast progression upon diagnosis. Despite the developments in diagnosis and treatment techniques and the use of post-surgery adjuvant treatment combinations in local diseases, the disease may still progress [2]. Gastric cancer is a biologically heterogeneous disease with many genetic and epigenetic variations. Despite this heterogeneity of the illness, patients in same stages received similar treatments. This changes as transtuzumab shows survival advantages in patients with metastatic gastric cancer [3]. Treatment choice is considered according to the biology of the disease.

Prognosis of the disease can be estimated by particularly clinical and laboratory parameters that are determined during the diagnosis. Determination of the mechanisms playing a part in oncogenesis in patients can bring forward the treatments towards the target. Laboratory parameters can be used for the determination of treatment intensity and type to be applied to patients.

The HER2 (ERBB2) is a transmembrane protein of 185kD weight having a tyrosine kinase activity. It is a member of the epidermal growth factor receptor family and is synthesized by *cerb-B2* which is a proto-oncogene. Activation of HER2 receptors leads to the, the activation of cellular signal transduction systems, resulting in the cellular transformation and cell proliferation events associated with cancer. Overexpression and gene amplification of HER2 have been detected in the development and progression of many cancers and studied in breast cancer more [4;5]. HER2 amplification is observed in 15–25% of all breast cancer cases [6;7]. Rate of HER2 expression in gastric cancer shows a wide variety in literature. By immunohistochemical method expression rate was found as %6.8-%34, and %7.1-%42.6 by Fluorescence in situ hybridization (FISH) method [8]. In first step of the treatment of metastatic gastric cancer therapies targeting HER2 are used in daily clinical practice. Therefore it is important to know the rate of HER2 expression in patients with gastric cancer. In this study we examined the rate of HER2 expression in patients with gastric cancer, by immunohistochemical method.

Material and Method

The Patient Group

A total of 50 patients with gastric adenocarcinoma who underwent diagnosed at Antalya Education and Research Hospital from 2008 to 2011 were enrolled in this study. By retrospective analysis of patients' data, stage of the disease and clinicopathological information were obtained. Patients whose treatment were started in an other hospital and continued in our hospital were not included in this study.

Immunohistochemical studies

Tumor samples obtained right after the surgery or endoscopy were fixed in 10% formaldehyde. After fixation, tumor samples were embedded in paraffin. Then, histologic sections with a 4 µm thickness were obtained from paraffin blocks and were initially stained with hemotoxylin- eosine for assessment.

The histologic sections were de-paraffinized in incubators at

60°C for one hour. Afterwards, they were kept in xylene for 10 minutes and in 100% alcohol for 5 minutes and then washed in water. Slides were kept in solution buffered with 10% citrate solution in microwave at maximum power (800 watts) for 15 minutes. Then the power was decreased to the half and they were kept in the microwave for another 20 minutes. Slides taken out of the microwave were kept in room temperature for 20 minutes. Endogenous peroxidase activity was removed by being kept in 3% hydrogen peroxide for 20 minutes. Slides washed in distilled water were treated with 3x5 PBS and protein blockage was dripped on them. Five minutes later, Her-2 antibodies were dripped on the slides without washing off the blockage. After being kept in primer antibody for 30 minutes, they were taken into PBS and washed for 5 minutes. Afterwards, they were treated with biotinylated secondary antibody for 20 minutes and washed in PBS for 5 minutes. They were kept with peroxidase conjugate antibody for 20 minutes. Then they were washed in PBS for 5 minutes. They were kept in chromogenous (DAB) for 5 minutes. Slides washed under tap water were adversely stained with haematoxyline. They were dehydrated, dried and mounted with entellane.

For the staining of the samples Her-2, lyophilized monoclonal mouse antibody (clone e2-4401+ 3B5, 1:600, Thermo Science, Fremont, USA) were used.

Immunohistochemical Scoring

Expression of HER 2 in tumor cells was evaluated by a pathologist unaware of the clinical information of the patients, under an olympus CX41 microscope. Immunohistochemical scoring was made according to the scoring system suggested by Hofmann et.al as "Consensus panel recommendations on Her2 scoring for gastric cancer" [9]. Ccases with a 2+ /equivocal score were accepted as negative since we were not able to apply FISH testing.

Statistical analyses

Statistical analyses were performed using the SPSS software version 15. TNM stage, lymph node status, distant metastasis and being over or under 60 years of old were analysed by crossing tables in Her2 positive and negative groups. The Chi-square test or Fisher's exact test, where appropriate, was used to compare these proportions in different groups. The P-values less than 0.05 were considered to be statistically significant.

Results

This study involved a total of 50 patients consisting of 17 (34%) females and 33 (66%). The mean age of the patients was found as 62±10,7. The most commonly observed complaint was abdominal pain determined in 15 (30%) of the patients and loss of weight and dysorexia followed with a decrease in frequency. It was determined that in 19 (38%) of the patients diagnosis of metastatic disease was made by biopsy and imaging methods and the rest of the patients underwent surgery. Twelve (24%) of the patients had a liver metastasis, liver was considered as the most frequent location for metastasis. Peritoneal metastasis was observed in 5 patients whereas 4 (8%) of the patients had a synchronous metastasis of liver and periton.

In 64% of the patients ECOG score was determined as 0 and

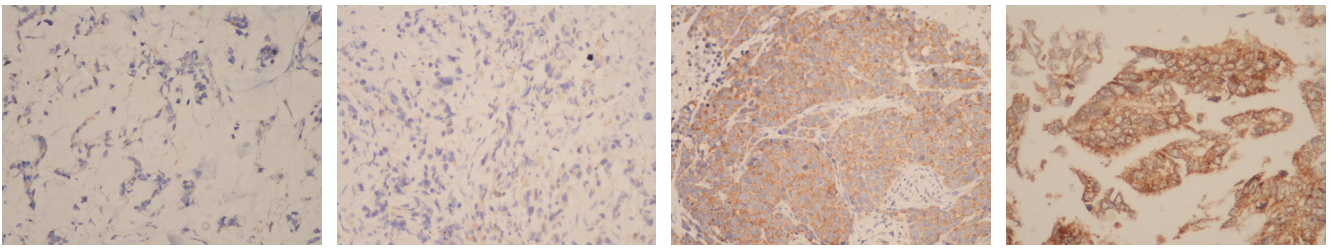


Figure 1. No staining (score 0), HER2 x200
 Figure 2. Weak and incomplete membrane staining of tumor cells (score 1+), HER2 x200
 Figure 3. Complete membrane staining at least 10% of tumor cells, nonuniform and weak intensity (score 2+), HER2 x200
 Figure 4. Uniform intense membrane staining of >30% of tumor cells (score 3+), HER2 x400

the rest of the patients established an ECOG score of 1. When grouped according to stage ; 3 patients (6%) were in stage I, 7 patients (14%) were in stage II , 11 patients (22%) were in stage III and 24 (48%) patients were in stage IV.

HER-2 protein status in 50 gastric carcinoma tissue samples was determined with immunohistochemical staining. Of the 50 gastric carcinoma tissue samples, 25 (50%) were scored as 0, 11 (22%) as 1, 7 (14%) as 2, and 7 (14%) as 3 (Figure 1-4). The positive rate was approximately 14% (7/50). The HER-2 status was not correlated with the TNM stage, lymph node status, distant metastasis and age (p:0,344, p:0,315, p:0,181, p:0,96). The HER-2 status was correlated with sex (p:0,041). All of the Her-2 positive patients were male (Table 2).

Table 1. General characteristics of patients

	Mean,Standard Deviation	Median
Age	62±10,7	62,5
AST (U/L)	21,5±8	20
ALT (U/L)	22,5±15,6	18
ALP (U/L)	181,8±28.1	93
LDH (U/L)	218,3±70,9	190
WBC (103/mm ³)	5.6±0,8	5.6
HGB (g/dl)	9,5±1.4	10.9
PLT(103/mm ³)	329,3±47.1	320

Table 2. Patient groups according to Her-2 status

	Her2 Negative	Her 2 Pozitive	P Value
Gender			
Kadin	17 (39,5%)	0	p:0.041
Erkek	26 (60,5%)	7(100%)	
Age	63±10.4	61±13.2	p:0.96
Stage			
Stage 1	3 (7%)	1(14.3%)	p:0.344
Stage 2	7 (16.3%)	0	
Stage 3	13 (30.2%)	1(14.3%)	
Stage 4	20 (46.5%)	4(71.4%)	
Lymph Node Status			p:0.315
Metastatic	35 (%81.4)	7 (100%)	
Nonmetastatic	8 (%18.6)	0	
Distant Metastasis			p:0.181
Negative	23 (53.5%)	2 (28.6%)	
Pozitive	20 (46.5%)	5 (71.4 %)	

Discussion

Gastric carcinoma is a disease entity with aggressive progression, exhibiting differences in epidemiologic and clinical profile

worldwide. It accounts for approximately 8% of all new cancer cases [10]. It occupies the second place as the cause of death by cancer [11]. In Turkey, it is the second most-frequently seen form of cancer in men, and the third most-frequently seen in women [12].

In this study, we have identified the HER2 expression ratio in metastatic gastric cancer as 18.8% by IHC. Yano et al. found this ratio as 27% using FISH method in 200 Japanese patients who underwent surgery for gastric tumor [13]. Tanner et al. identified HER2 amplification in 12% of 131 cases of gastric adenocarcinoma using CISH method, and in 24% of 100 cases with gastroesophageal junction tumor [14].

In the widest range of study on HER2 expression, 3883 patients with advanced stage gastric cancer were evaluated and HER2 positivity was found as 22.9%. In this study, there was not any difference between European and Asian countries. Intestinal type of HER2 positivity was found at a higher ratio than the diffuse/mixed type. In gastroesophageal junction tumors, HER2 positivity was identified at a higher rate than the gastric tumors [15].

Determination of prognostic factors in gastric cancer has an important role in estimating the survival of the patients and determining the treatment method. In many studies the depth of invasion and lymph node metastasis of the tumor are demonstrated to be important prognostic factors [16;17]. Parameters such as the depth of invasion of the tumour (T), local lymph node metastasis (N) and distant metastasis (M) are used in TNM staging. The studies show that patients in the same pathological stage may have different prognosis. Many biological reagents are observed in order to determine these different prognostic groups [18;19].

Our study shows that, HER-2 status was not correlated with the TNM stage, lymph node status, distant metastasis and age. Yan et al found the rate of Her-2 expression as 15.2% in their study, similiar to the rate we found. They found a significant correlation between Her-2 expression and TNM satge, lymph node metastasis and distant metastasis in their study. In our study we found a relation betwwen sex and Her-2 expression while no such relation was found in the study of Yan et. Al [20]. Our study consisted of 17 female patients, therefore we think that the reason of this difference may be the small number of the patients of our study. The correlation between the Her2 status and TNM stage, lymph node and distant metastasis had been searched in many studies. Different results were obtained in these studies and in some of them a significant correlation was found whereas some established no relationship [20-22].

In our study only IHC method was performed and patients who

had a score of 2+ were considered to have negative HER2 expression. It is known that some of the patients with breast cancer with a score of 2+ established HER2 expression by FISH method. Therefore, we think that HER2 expression ratio may differ from the values we have obtained.

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