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PROGNOSTIC SIGNIFICANCE OF HER-2 EXPRESSION IN GASTRIC CANCER

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Abstract: In gastric cancers we purposed to examine the relationship between age, gender, invasion depth, lymph node metastasis, lymphovascular invasion, Lauren's classification, presence of perineural invasion and the expression of Human Epidermal Growth Receptor (HER-2) by immunohistochemical (IHC) and dual-color silver-enhanced in situ hybridization (D-SISH) method. In our study, 242 gastric resection materials that were studied HER-2 by IHC and/or D-SISH method referred to our department between January 2014 and December 2017 for malignancy were included. The relationship between HER-2 expression rate and prognostic parameters was investigated. No statistical relation was found between age and gender and HER-2 positivity in either method. No statistically significant results were obtained between lymphovascular invasion, lymph node metastasis, presence of perineural invasion, depth of invasion and Lauren classification, and HER-2 positivity by immunohistochemical and D-SISH method. In our study, no statistically significant results were obtained between important prognostic parameters and HER-2 positivity with either IHC or D-SISH method. In 24.1% of the cases that were HER-2 positive by IHC, positivity was not observed with the D-SISH method. Also, 20.8% of the cases reported as immunohistochemically uncertain (equivocal) were seen as positive by the D-SISH method. Based on all these results, we would like to state that HER-2 positivity / negativity determined by IHC can be changed by the D-SISH method. In addition, although it was shown in our study that HER-2 has no prognostic significance in gastric carcinomas, it is reported in studies in the literature that the addition of Trastuzumab to chemotherapy increases survival. For this reason, we think that it would be more beneficial to apply the IHC and D SISH method together to obtain the true HER-2 result.

Keywords: Gastric cancer, Prognosis, HER-2

1. Introduction

According to World Health Organization (WHO) data, gastric cancer ranks the fifth among all malignancies with a rate of 6.8% in both sexes in worldwide. It ranks fourth with 8.5% for men and fifth with 4.8% for women (Bernard, 2014). In our country, according to the latest data of Cancer Department, it ranks sixth in total for both sexes (Gültekin and Boztaş, 2014). In recent years, there has been a serious increase in the frequency of gastric cancer (Alacali, 2012). The frequency of gastric cancer changes depending on the geographies. For example, in Japan, Chile, Costa Rica and Eastern European countries, its frequency is much higher than other countries. It also includes geographical differences within the country. Its frequency is higher in Eastern Anatolia Region than other regions in our country (Göçmen and Kocaoğlu, 2000). According to the WHO report; cancers are the cause for annually 7.6 million deaths worldwide and almost 736 000 of them are due to gastric cancer (Msyamboza et al., 2012). In cancer-related death gastric cancer ranks second in men and third in women. This ranking is similar in our country (Yalçın et al., 2006). It is extremely important to determine the prognosis of this malignancy, which has an extremely high mortality and morbidity,

and biomarkers associated with the prognosis.

The human epidermal growth receptor [C-erbB-2 (HER-2)] is a member of the Her2 / neu family, closely related to growth factor receptors and is located on chromosome 17 (q21). It regulates cell growth, proliferation and survival (Yildirim et al., 2012). HER-2 gene amplification or increased protein expression in gastric cancers is found positive in a wide range of 7-34% of primary tumors (Uprak, 2014). While HER-2 positivity is considered as a bad prognostic criterion in breast cancers, the prognostic importance of HER-2 positivity is still controversial in gastric cancer (Uprak, 2014). However, the addition of Trastuzumab, which is a drug used in patients with HER-2 positivity in advanced gastroesophageal junction and gastric cancers, has been indicated to increase the survival rate (Tanner et al., 2005). We aimed to investigate the relationship between the HER-2 expression and the major prognostic parameters such as the depth of invasion, lymph node metastasis, lymphovascular invasion, the Lauren classification, and the presence of perineural invasion by immunohistochemical (IHC) and dual-color silver-enhanced in situ hybridization (DISH) methods in gastric cancer.

2. Material and Methods

Two hundred forty-two gastric resection materials referred to our department between January 2014 and December 2017 for malignancy were included in the study. In 165 of the cases, the IHC score was HER-2 negative (Score 0 and Score 1). Only in 1 of these cases HER-2 was studied with D-SISH method. In addition, HER-2 gene expression was studied by D-SISH method in 77 cases determined to be HER-2 positive and uncertain (equivocal) by IHC and these results were compared (D-SISH was studied in 78 cases in total). Twenty of the cases who underwent D-SISH were gastric resection (25.6%) and 58 (74.4%) were the first biopsy specimens of these resection materials. Biopsy materials were used as the first choice for performing D SISH. Resection materials were used in cases where biopsy materials were not available. Gastric resection and biopsy materials of the cases were examined retrospectively. The clinicopathological data of the cases were taken from the pathology archive. Sections from the blocks where the tumor was the most intense were investigated by immunohistochemical (IHC) and D-SISH methods. Histopathological diagnoses, Her-2/neu scores obtained by IHC and D-SISH method were reevaluated by two pathologists.

The IHC dyeing characteristic for the HER-2 ratio was defined for resection materials as follows: Score 0: in <10% of cancer cells have no reactivity or membranous reactivity, Score 1: weak or barely perceptible membranous reactivity in ≥10% of cancer cells; Score 2: weak to moderately complete or non-persistent membrane reactivity in ≥10% membranous tumor cells; Score 3: strong complete, persistent membranous reactivity in ≥10% of cancer cells.

The IHC dyeing characteristic for the HER-2 ratio was defined as follows for biopsy materials: Score 0: no reactivity in cancer cells or no membranous reactivity; Score 1: poor membranous reactivity in tumor cells; Score 2: moderately intact membrane reactivity in tumor cells; Score 3: severe, uninterrupted membranous reactivity in tumor cells (Bang et al., 2010) (Figure 1). In terms of the IHC, the ones with HER-2/neu Score 0 and 1 were considered as negative, with Score 2 was considered as equivocal, with Score 3 was considered as positive.
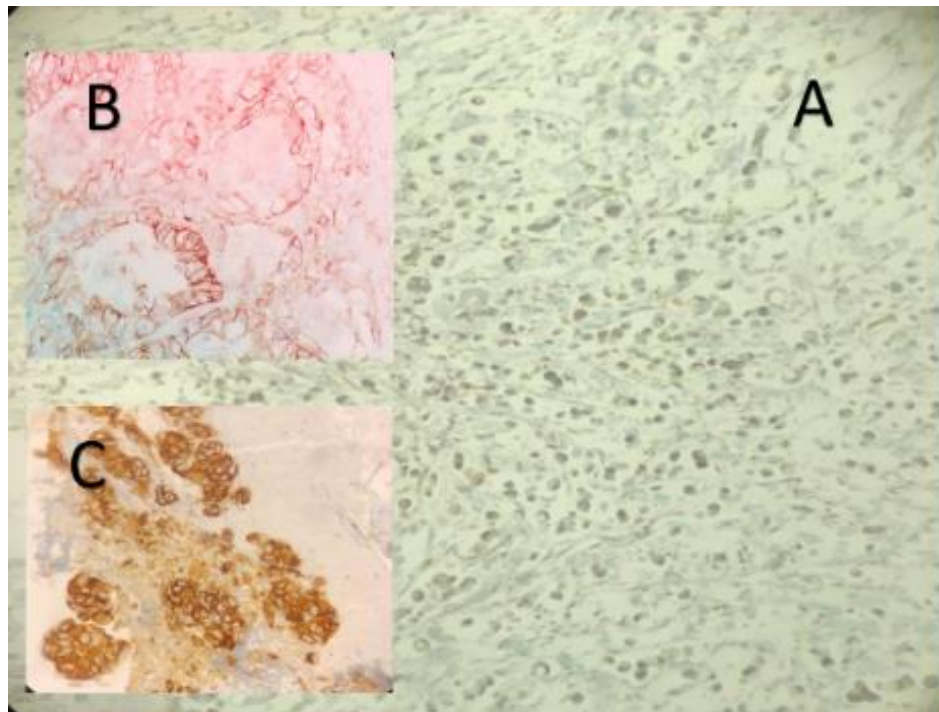

Figure 1. HER 2 by IHC method (A= score 1+ (negative), B= score 2+ (equivocal), C= score 3+ (positive)).

D-SISH results are expressed as the ratio between the HER2 gene and the number of chromosome 17 counted in nuclei in 20 cancer cells. Cases with a HER2 / Chromosome 17 ratio of <1.8 were considered negative for HER2 gene amplification, while those with a HER2 / Chromosome 17 ratio> 2.2 were considered positive for HER2 gene amplification (Koh, Lee, Lee, Kang, & Gong, 2011) (Figure 2).

The relationship between HER-2 expression detected by IHC and D-SISH methods and gender, age, lymphovascular invasion, depth of invasion, lymph node metastasis, Lauren classification, perineural invasion was investigated.
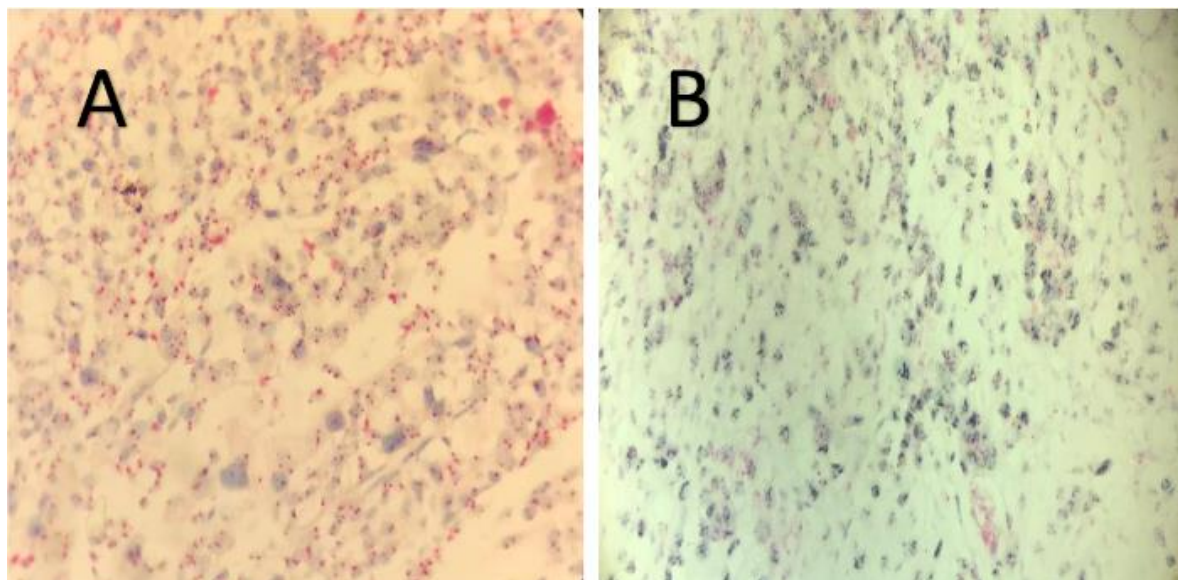
Figure 2. HER 2 by DSISH method (A= negative, B= positive).

2.1. Statistical Analysis

Statistical analysis was done by using IBM SPSS20 statistics analysis program. Data were presented as standard deviation, mean, minimum, maximum, median percentage and number. The normal distribution of continuous variables was examined with the Shapiro Wilk-W test when the sample size was <50 and the Kolmogorov Simirnov test when it was >50. In the comparisons between two independent groups, the Independent Samples t test was used when the normal distribution condition was met, and the Mann Whitney u test was used when it was not provided. In 2x2 comparisons between categorical variables, the expected value (>5) was made using the Pearson Chi-square test, the expected value (3-5) using the chi-square yacht test and the expected value (<3) using the Fisher's Exact test. The consistency of the newly developed diagnostic test with the clinical gold standard was evaluated by calculating Cohen's Kappa coefficient.

2.2. Ethical Consideration

The study was approved by Atatürk University local ethics committee with the number 2020/ B.30.2.ATA.0.01.00.

3. Results

The average age of 242 cases in our study was 60 ± 12, the smallest age was 22 and the oldest was 94. There was no significant difference between the mean ages in both sexes (P = 0.052). 165 (68%) of the cases were male and 77 (32%) were female. Fifty-four of the patients who underwent D-SISH were male (69.2%) and 24 were female (30.8%) and the male/female ratio was 2.25. Twenty of the patients who underwent D-SISH were gastric resection (25.6%) and 58 were biopsy samples (74.4%). According to the Lauren calcification, 6.8% of our cases were diffuse and 93.2% were intestinal type. In terms of the IHC, the HER-2 results of the 48 of our

cases were equivocal (Score 2), 29 of them were (12%) positive (Score 3), 165 of them were negative (Score 0 and Score1). 41% of the cases treated with HER-2 by the D-SISH method were positive. When the HER-2 positivity of the cases was compared, IHC and D-SISH methods were seen to be consistent (P < 0.01). When the cases whose HER-2 results were uncertain (Equivocal) with the IHC method analyzed, it was observed that 20.8% them were positive and 79.2% them negative with the D-SISH method. 75.9% of the cases with positive HER-2 results with IHC were also followed up with D SISH method. The cases with negative HER-2 results by the IHC was also negative with the D-SISH method (Table 1).

Table 1. Comparison of HER-2 values with IHK method and D SİSH method

HER-2 (IHC)	HER-2 (D-SISH)			
	Negative		Pozitive	
	n=46	%	n=32	%
Score 1	1	2.2	0	0.0
Score 2	38	82.6	10	31.3
Score 3	7	15.2	22	68.8

HER-2= human epidermal growth factor receptor 2.

There was no statistical relationship between age and gender and HER-2 positivity by IHC method (P = 0.684 / P = 0.574). There was no statistically significant result between the HER-2 positivity by IHC method and the prognostic parameters such as lymphovascular invasion (P = 0.399), lymph node metastasis (pN value) (P = 0.2847), presence of perineural invasion (P = 0.2847), depth of invasion (P = 0.163) and Lauren classification (P = 0.3163).

There was no statistical relationship between age and gender and HER-2 positivity by D-SISH method (P = 0.783 / P = 0.675). No statistically significant results were obtained between HER-2 positivity by D-SISH

method and the prognostic parameters such as lymphovascular invasion (P = 0.7032), lymph node metastasis (pN value) (P = 0.1667), presence of perineural invasion (P = 0.8981), depth of invasion (P = 0.154) and Lauren classification (P = 0.6470) (Table 2).

Table 2. Relationship between HER-2 positivity by IHC and DSISH method with age, gender lymphovascular invasion, lymph node metastasis, perineural invasion, depth of invasion, Lauren classification

HER-2	D-SISH (P value)	IHC (P value)
Age	0.7830	0.6840
Gender	0.6750	0.5740
LV	0.7032	0.3990
LN	0.1667	0.2847
PI	0.8981	0.2847
DI	0.1540	0.1630
LC	0.6470	0.3163

IHC= immunohistochemical, LV= lymphovascular invasion, LN= lymph node metastasis, PI= perineural invasion, DI= depth of invasion, LC= Lauren classification.

4. Discussion

Gastric cancers are one of the most common malignancies in worldwide, most patients are advanced at the time of diagnosis and there are metastases (Uprak, 2014). It is important to develop new and effective treatment modalities because of the survival rates as low as 20% (Ross et al., 2003). In recent years, it has been reported that combined chemotherapeutic agents increase survival in gastric cancer (Cunningham et al., 2005). The development of targeted therapies in gastric cancers has also provided an advantage in patients' life expectancies. HER-2 positivity has been demonstrated in different types of cancer such as head and neck, endometrium, colon, ovary, bladder, lung, uterus, cervix, esophageal and gastric carcinomas. For a long time, HER-2 positivity has been analyzed as a routine in breast carcinomas. It is positive in approximately 34% of cases in breast cancers and is associated with aggressive behavior, poor response and resistance to chemotherapeutics (Allgayer et al., 2000). The prognostic importance of HER-2/neu positivity in gastric cancers is still controversial (Luis et al., 2013).

Most of our patients are male and the youngest age is 22, the highest age is 94, the average age is 60 and it is compatible with the literature (Yalçın et al., 2006). There was no statistical relationship between age and gender and HER-2 positivity detected by IHC and D-SISH methods. Kim et al. (2011) report that HER-2 positivity is more common in older age, males and intestinal type tumors. However, in most of the studies in the literature, there was no significant relationship between HER-2 positivity and age and gender (Grávalos et al., 2011).

In our study, HER-2 positivity was observed in 12% of

our cases by IHC. HER-2 positivity rate in gastric carcinomas is reported by 4-44% with the IHC method. In this respect, our determination is compatible with the literature (Hofmann et al., 2008). In our study, no statistically significant results were obtained between the HER-2 positivity by IHC or D-SISH methods and prognostic parameters such as lymphovascular invasion, lymph node metastasis [pN value], presence of perineural invasion, depth of invasion and Lauren classification. Different results have been obtained in studies examining the link between HER-2 positivity and prognostic parameters such as organ metastasis, lymph node metastasis, and depth of invasion, lymphovascular invasion, and Lauren classification. In their studies, Yonemura et al. (1991) found a significant relationship between HER-2 expression and macroscopic tumor diameter, depth of invasion, lymphovascular invasion, and lymph node metastasis. Fornaro et al. (2011) reported that intestinal-type gastric cancers show more HER-2 positivity compared to diffuse-type gastric cancers. In their study, Junior et al. (2016) reported that HER-2 overexpression was a poor prognostic factor in metastatic gastric cancer patients. In their meta-analysis study, Lei et al. (2017) stated that genetic and geographic factors were effective in HER-2 expression, and HER-2 positivity was associated with gender, TNM stage, distant organ metastasis, lymph node metastasis, and Lauren classification.

Although there are many studies reporting HER-2 expression as a bad prognostic factor in the literature, there are also many studies stating that it is not prognosticaly significant. In their study, Sasanno et al. (1993) did not find a relationship between HER-2 positivity and the depth of invasion, histological grade, clinical stage, and lymph node metastasis which are important prognostic parameters. In the studies of Uprak (2014) similar to our study, no relation was found between HER-2 positivity and tumor diameter, lymph node metastasis, depth of invasion, distant organ metastasis, lymphovascular invasion, perineural invasion, and tumor stage.

HER-2 positivity can be evaluated by the IHC and HER-2 amplification can be evaluated by in-situ hybridization method. In the D-SISH method, which has been used recently, the probe specific for the HER-2 gene region is marked with silver. The centromeric region of chromosome 17 becomes visible with red chromogen (Diniz Ünlü et al., 2015). When these two methods are compared with each other, the IHC method has advantages such as providing feedback as soon as possible, being affordable and relatively simple in the evaluation of HER-2 expression (Koh et al., 2011). Another controversial point is the determining the initial method for the evaluation of the HER-2 evaluation. Currently, there is no single "gold standard" to determine HER-2 positivity/negativity. In addition to those who argue that the first choice should be the IHC method as it is an easier and cheaper method, there are also those

who state that it is not wrong to start with D-SISH (Diniz Ünlü et al., 2015; Wolff et al., 2014).

In our study, the D-SISH method was 20.8% positive for where HER-2 results were uncertain (equivocal) by the IHC. Only 75.9% of the cases with positive IHC HER-2 results were also positive by the D-SISH method. In other words, 24.1% of the cases that were positive for IHC did not show positivity with the D-SISH method. Therefore, it was found that more effective results were obtained when both methods were applied in combination. The ASCO/CAP study group also reports that approximately 20% of current HER-2 tests may be false positive/negative relative to preanalytical and analytical variables (Diniz Ünlü et al., 2015). Based on all these results, we would like to state that HER-2 positivity / negativity determined by IHC can be changed by the D-SISH method. In addition, although it was shown in our study that HER-2 has no prognostic significance in gastric carcinomas, it is reported in studies in the literature that the addition of Trastuzumab to chemotherapy increases survival. For this reason, we think that it would be more beneficial to apply the IHC and D SISH method together to obtain the true HER-2 result.

5. Conclusion

In our study, no statistically significant results were obtained between lymphovascular invasion, lymph node metastasis, presence of perineural invasion, depth of invasion and Lauren classification, and HER-2 positivity by IHC and D-SISH method.

Author Contributions

OC and SÖ originally conceived the idea and hypothesis. SÖ designed the study. OC made the research organization. SÖ collected the data. OC interpreted the results. OC and SÖ drafted the manuscript. All authors reviewed and approved the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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