Human epidermal growth factor receptor 2 (HER-2) status evaluation in advanced gastric cancer using immunohistochemistry versus silver *in situ* hybridization

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ABSTRACT

Accurate identification of human epidermal growth factor receptor 2 (HER-2) status in advanced gastric cancer patients is of utmost importance in terms of treatment planning. This study aimed to examine the HER-2 status in advanced gastric cancer patients using both immunohistochemistry (IHC) and silver *in situ* hybridization (SISH) techniques and to investigate concordance and diagnostic accuracy. In addition, associations between clinical parameters and HER-2 status were examined. A total of 313 patients diagnosed with locally advanced (Stage III: T3-4, N+) recurrent or metastatic adenocarcinoma of the stomach or esophagogastric junction, between 2009 and 2015, were included. HER-2 status was examined using both IHC and SISH techniques and the findings were compared. Overall SISH-confirmed HER-2 positivity rate was 22%. Multivariate analysis identified only well-differentiated tumor as a significant predictor of HER-2 positivity (OR: 2.9, 95% CI: 1.4-5.9, $p = 0.003$). When IHC 2+ and 3+ were considered positive for HER-2 status, sensitivity, specificity, and concordance rate (κ) was 95.7%, 93.8%, and 0.84, respectively. Corresponding figures when only IHC 3+ cases were considered positive were lower: 50%, 100%, and 0.61, respectively. The present method used for the identification of HER-2 positive gastric cancer patients provides satisfactory results. However, better categorization of IHC 2+ cases has the potential to improve the diagnostic accuracy, which is particularly important when more sophisticated methods are not readily available.

KEY WORDS: Human epidermal growth factor receptor 2; gastric cancer; immunohistochemistry; silver *in situ* hybridization
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INTRODUCTION

Gastric carcinoma is a common malignancy, and the second leading cause of death from cancer worldwide [1]. Gastric cancer is a biologically and genetically heterogeneous tumor. A substantial proportion of gastric cancer cases is diagnosed at an advanced inoperable or metastatic stage [2] and require palliative treatment.

The human epidermal growth factor receptor 2 (*HER-2*) oncogene encodes a transmembrane receptor glycoprotein, which has tyrosine kinase activity. Overexpression of the receptor protein or amplification of the gene has been shown in a number of malignancies including gastric cancer [3,4]. HER-2 protein has a role in the initiation/progression of cancer and intracellular signaling and its overexpression has been associated with poor prognosis [5]. A recent study showed that inoperable or advanced metastatic gastric cancer patients with HER-2 overexpression could benefit from the treatment with anti-HER-2 monoclonal antibodies (trastuzumab) [6]. Thus, accurate identification of this subset of patients has gained importance in terms of the treatment planning.

To date, several techniques have been introduced to test HER-2 status, immunohistochemistry (IHC) and *in situ* hybridization (ISH) techniques being more commonly utilized. Satisfactory concordance rates have been shown between these two techniques [7]. The common practice is to initially perform immunohistochemical evaluation, where 0/1+ cases are considered negative and 3+ cases are considered positive for HER-2 overexpression. On the other hand, 2+ cases are accepted as equivocal and confirmation with more sophisticated methods is necessary.

This study aimed to examine the HER-2 status in advanced gastric cancer cases using both IHC and silver *in situ* hybridization (SISH) techniques and to investigate the diagnostic value of IHC in predicting SISH-confirmed HER-2 status.

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In addition, associations between HER-2 status and clinical parameters were examined.

MATERIALS AND METHODS

Patients

A total of 313 patients diagnosed with locally advanced (Stage III: T3-4, N+) recurrent or metastatic adenocarcinoma of the stomach or esophagogastric junction, between 2009 and 2015, were included in this study. One hundred and thirty cases were diagnosed in our institution (Istanbul University Cerrahpasa Medical Faculty), and 183 cases were consultation cases sent to our department for HER-2 evaluation from several medical centers in Istanbul. The diagnosis was established with the histopathological evaluation of endoscopic biopsy or surgical resection material. The consultation cases were also reviewed, and the diagnoses were confirmed. The consent of all patients was obtained at the time of referral to our hospital. The clinical and demographic features were retrieved from the medical records of each patient. The tumors were evaluated histologically as follows: Well-differentiated tumors included grade I and grade II adenocarcinomas. Poorly differentiated group, on the other hand, consisted of grade III adenocarcinomas, mucinous adenocarcinomas, and signet ring cell carcinomas. For the purpose of HER-2 status evaluation, 4-micrometer sections were obtained from the archived paraffin blocks (for each patient, 1 section for IHC, 2 sections for SISH were obtained).

In the routine practice, IHC is usually used as the initial test, 0/1+ cases are considered HER-2 negative, and 3+ cases are considered HER-2 positive. Equivocal cases (IHC 2+), on the other hand, are candidates for an additional ISH test to confirm HER-2 gene amplification. In our study, IHC and SISH were applied to all cases (n = 313) (Figure 1).

Immunohistochemistry

IHC examination was performed using a Ventana Benchmark XT automated staining system and c-erbB2 (clone 4B5) (Ventana, Tucson, AZ, USA). ToGA criteria were used for scoring between 0 and 3+. The details of the criteria are given in Table 1 [6]. In general, HER-2 2+ results are considered equivocal and demand an ISH test for the conclusion of the final HER-2 amplification status. In our study, the diagnostic value of two different scenarios was tested as an indication of HER-2 status: 2+ and 3+ were considered positive or only 3+ was considered positive.

Silver *in situ* hybridization

For SISH examination, Ventana Benchmark CT automated system, Ultraview SISH detection kit, inform Her2 DNA probe, and inform chromosome 17 DNA probe (Ventana, Tucson, AZ, USA) were used. Using slides prepared for c-erbB2 and chr17, signals in a total of 40 cell nuclei were counted at ×1000 magnification from the site of the tumor. If the mean number of signals for c-erbB2 divided by the mean number of signals for chr17 was >2, the sample was considered positive for HER-2 (c-erbB2) amplification.

Statistical analysis

For the analyses of data, SPSS version 21 (SPSS Inc., Chicago, IL, USA) was used. Normality was tested using the Kolmogorov-Smirnov test and graphical methods. For the comparison of continuous variable (age), the Mann-Whitney U test was used. Categorical variables were compared using Pearson's chi-square test. Logistic regression was used for multivariate analysis to identify the independent clinical predictors of HER-2 positivity. A value of $p < 0.05$ was considered statistically significant. Diagnostic parameters (sensitivity, specificity, positive predictive value, negative predictive value) for predicting SISH-confirmed HER-2 status were calculated, and the two methods were compared using 95% confidence interval. In addition, kappa statistics were calculated for the concordance between the methods.

RESULTS

Table 2 shows patient characteristics and the comparison of HER-2 negative and positive patients (as assessed by

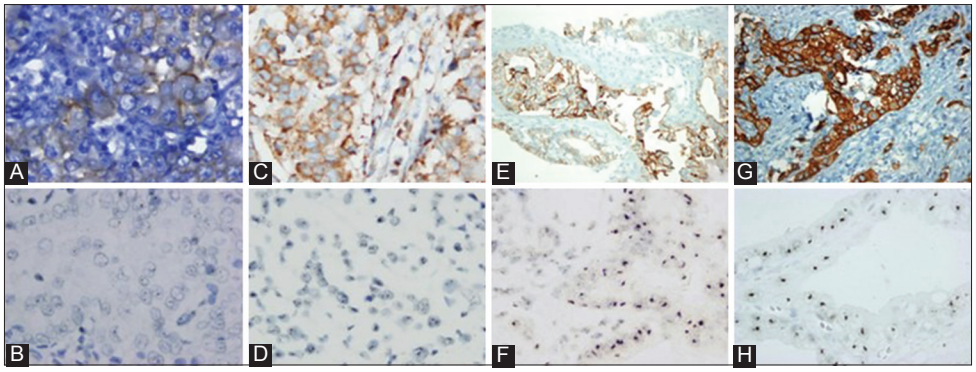

FIGURE 1. (A and B) Case with immunohistochemistry (IHC) score (0), silver *in situ* hybridization (SISH) (-), (C and D) Case with IHC score (1+), SISH (-), (E and F) Case with IHC score (2+), SISH (+), (G and H) Case with IHC score (3+), SISH (+).

TABLE 1. Immunohistochemistry scoring for HER-2 gastric and gastroesophageal junction cancers, by type of diagnostic specimen

HER-2 score	Surgical specimen staining pattern	Biopsy specimen staining pattern	HER-2 overexpression assessment
0	No reactivity or membranous reactivity in<10% of tumor cells	No reactivity or no membranous reactivity in any tumor cells	Negative
1+	Faint or barely perceptible membranous reactivity in≥10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in≥10% of tumor cells	Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in≥10% of tumor cells	Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Positive

HER-2: Human epidermal growth factor receptor 2

TABLE 2. Comparison of HER-2 negative and positive* gastric cancer patients treated from 2009 to 2015 with regard to demographic and clinical characteristics

Demographic and clinical characteristics	All patients (n=313)	HER-2 negative (n=243)	HER-2 positive (n=70)	*p* value
Age, years (mean±SD)	60.2±11.0	59.7±11.3	61.8±9.7	0.315
Male gender (%)	213 (68.1)	161 (66.3)	52 (74.3)	0.204
Sampling method (%)				
Endoscopic	156 (49.8)	118 (48.6)	38 (54.3)	0.399
Surgical	157 (50.2)	125 (51.4)	32 (45.7)	
Differentiation‡ (%)				
Well-differentiated	212 (67.7)	153 (63.0)	59 (84.3)	0.001
Poorly differentiated	101 (32.3)	90 (37.0)	11 (15.7)	
Location† (%)				
Cardia	75 (24.5)	51 (21.5)	24 (54.8)	0.05
Corpus	91 (29.7)	77 (32.5)	14 (20.3)	
Antrum	117 (38.2)	89 (37.6)	28 (40.6)	
Diffuse	23 (7.5)	20 (8.4)	3 (4.3)	

Unless otherwise stated, data presented as n (%). *As assessed by silver *in situ* hybridization method. †Metastatic lesions not included. ‡Well-differentiated tumors include grade I or II adenocarcinomas with or without accompanying neuroendocrine, signet ring, or mucinous component. Poorly differentiated tumors include grade III adenocarcinomas, signet ring cell carcinomas, and mucinous adenocarcinomas. HER-2: Human epidermal growth factor receptor 2

SISH). Overall, the SISH-confirmed HER-2 positivity rate was 22%. The IHC and SISH positivity rates are shown in Table 3. One hundred and eighty-seven of 313 cases were negative for HER-2 with both IHC and SISH methods. In the univariate analysis, no association was found between the HER-2 positivity and age, gender, location, or sampling method. However, HER-2 positive tumors were more commonly well differentiated (p = 0.001). Similarly, the multivariate analysis identified only well-differentiated tumors as a significant predictor of HER-2 positivity (OR: 2.9, 95% CI: 1.4-5.9, p = 0.003).

Value of IHC in predicting HER-2 status

Table 4 shows the diagnostic value of two different interpretations of the IHC results for predicting SISH confirmed HER-2 positivity: 2+ and 3+ indicates HER-2 positivity versus only 3+ indicates HER-2 positivity. The first approach has much higher sensitivity with only 1% false negativity rate but slightly lower specificity with 5% false positivity rate. The second approach, on the other hand, has 11% false negativity rate but 0% false positivity rate.

TABLE 3. IHC and SISH positivity rates in gastric cancer patients treated from 2009 to 2015

Positivity rates	SISH+ (%)	SISH– (%)
IHC+++	35 (11.18)	0 (0)
IHC++	32 (10.22)	15 (4.79)
IHC+	0 (0.96)	41 (13.10)
IHC–	0 (0)	187 (59.74)
Total	70 (22.36)	243 (77.63)

IHC : Immunohistochemistry; SISH: Silver *in situ* hybridization

TABLE 4. Diagnostic performances of the two immunohistochemistry cut points in gastric cancer patients treated from 2009 to 2015

Parameter	2+ and 3+ indicates HER-2 positivity	Only 3+ indicates HER-2 positivity
Sensitivity	95.7 (87.2-98.9)	50 (18.0-27.5)
Specificity	93.8 (89.8-96.4)	100 (98.1-100)
Positive predictive value	81.7 (71.3-89.1)	100 (87.7-100)
Negative predictive value	98.7 (95.9-99.7)	87.4 (82.8-91)

Data presented as percentage (95% confidence interval). SISH confirmed (n = 70); HER-2 positivity: 2+ and 3+ indicates HER-2 positivity versus only 3 + indicates HER-2 positivity; HER-2: Human epidermal growth factor receptor 2.

The concordance rate (κ statistics) between SISH and IHC was 0.84 when 2+ and 3+ cases were considered HER-2 positive, and 0.61 when only 3+ cases were considered HER-2 positive.

DISCUSSION

HER-2 status of a locally advanced gastric carcinoma has a great clinical importance. The patients with false negative results could benefit from targeted therapy with trastuzumab but will not receive the therapy unless ISH is performed. On the other hand, patients with false positive results will have side effects from the drug but no clinical benefit. Furthermore, the cost of the drug is high.

This study examined the HER-2 status of the patients with locally advanced gastric cancer, several clinical characteristics, and the correlation between IHC findings and SISH findings in terms of HER-2 positivity. The main findings are the correlation between well-differentiated tumors and HER-2 positivity, and the high sensitivity and specificity of using both IHC 2+ and 3+ scores in predicting SISH confirmed HER-2 gene amplification.

This study found an overall HER-2 positivity rate of 22%, which is close to the findings of previous studies [5,8-10]. The association between well-differentiated tumors and HER-2 positivity found in this study is in line with the findings of Park et al., which found a significant association between HER-2 positivity and differentiated histology, both for localized disease and metastatic/recurrent disease [11]. In that study, elevated carcinoembryonic antigen levels, pulmonary metastasis, and distant lymph node metastasis have also emerged as significant predictors. Laboissiere et al. identified intestinal histological subtype, histologic grade, and the presence of lymphovascular invasion as significant predictors of HER-2 positivity in gastric cancer [12]. In a study from Japan, intestinal type, absence of peritoneal metastasis, and hepatic metastasis were significant independent factors related to HER-2 positivity [13]. In contrast, Ieni et al. found significant associations between HER-2 positivity and high grade, advanced stage, and high Ki-67 labeling index value [14]. Chen et al., on the other hand, could not find association between HER-2 positivity and any of the clinical pathological characteristics [15]. Although there seems to be tendency for HER-2 positive tumors to be more differentiated and of intestinal type, conflicting evidence may be explained by different study designs, patient populations, and assessment methods.

To date, a number of studies have tested the diagnostic value of IHC in detecting HER-2 positivity in gastric cancer in reference to more sophisticated molecular methods [7,9,16-23]. Most comprehensive data on the concordance between IHC and SISH methods comes from a recent meta-analysis, which included a total of 12,679 cases from 45 individual studies [7]. In that study, the concordance rate of HER-2 IHC 0/1+, 2+ and 3+ cases with ISH results was analyzed. Very high concordance rates were found between 0/1+ and 3+ IHC findings versus ISH results (0.969 and 0.915, respectively), whereas IHC 2+ findings showed very weak concordance with corresponding ISH results (0.393). The pooled sensitivity and specificity of IHC positivity (when both 2+ and 3+ were considered positive), in predicting ISH confirmed HER-2 positivity, were 0.86 and 0.91, respectively. The latter findings are in line with the findings of the present study. Although these findings indicate a high diagnostic accuracy of the IHC method, the authors emphasized the need for more detailed criteria for IHC 2+ cases to predict HER-2 gene amplification [7].

Another study examined HER-2 status of 122 equivocal gastric cancer cases (HER-2 IHC 2+) using FISH and found a very low concordance rate (13.9%), indicating the necessity of an ISH test for further categorization when IHC scores are 2+ [16].

Cho et al. compared four different IHC tests (HercepTest, A0485, 4B5, and CB11 antibodies) with FISH findings in terms of HER-2 positivity in gastric cancer patients [17]. IHC 2+ and 3+ cases were considered positive. A0485 had the highest sensitivity (86.5%) and also a high specificity (94.4%). On the other hand, CB11 had the highest specificity (98.4%) with an unsatisfactory sensitivity (0.60). All four IHC methods had a concordance rate higher than 0.93 with ISH results.

A recent study proposed a novel method combining IHC and dual-color SISH on one slide for the HER-2 evaluation in gastric cancer, which was able to evaluate both the gene amplification and protein overexpression status in the same cancer cell [18]. In that study, a relatively high concordance rate has been reported for IHC 2+ cases (95.83%; κ = 0.728).

The findings of the previous studies have already demonstrated low concordance of IHC 2+ score and ISH-confirmed HER-2 positivity, indicating the need for additional tests in these equivocal cases. However, the scenario considering both IHC 2+ and 3+ cases as positive has very high sensitivity and specificity. Only 1% of positive cases would be missed in the expense of 5% false-positive cases. On the other hand, considering only 3+ cases as positive would miss 11% of positive cases but without any false positives. Thus, IHC HER-2 2+ cases represent a subset of patients with uncertain diagnostic characteristics in terms of HER-2 status, which may be particularly caused by tumor heterogeneity. Therefore, IHC 2+ cases should be interpreted carefully and necessary confirmation tests should be carried out.

The results of this study showed that the present method used for the identification of HER-2 positive gastric cancer patients provides satisfactory results, given that HER-2 IHC 2+ cases are handled specifically and confirmation tests are

ordered to eliminate false positive findings. However, better categorization of IHC 2+ cases has the potential to improve the diagnostic accuracy of IHC, which is particularly important when more sophisticated methods (i.e. ISH) are not readily available.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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