RESEARCH ARTICLE

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HER2 Expression Status and Prognostic, Diagnostic, and Demographic Properties of Patients with Gastric Cancer: a Single Center Cohort Study from Iran

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Abstract

Background: The fourth most prevalent cancer worldwide and a major cause of death in developing countries is gastric cancer (GC). Human epidermal growth factor receptor 2 (*HER2*), is a proto-oncogene expressed in different solid tumors. This study aimed to evaluate possible associations of *HER2* expression status with survival rate, age, sex, tumor grade, histopathological type, and primary tumor location in patients with GC. **Methods:** Subjects were enrolled in this cohort study after consideration of inclusion and exclusion criteria. Biopsy specimens were stained using immunohistochemistry. Samples with a score of 3+ were considered to exhibit *HER2* overexpression. The mentioned variables were extracted from patients' files as well as by clinical evaluation. The Kaplan-Meier method was applied for analyzing the survival rate and Chi square for possible factor associations. **Results:** A total of 210 patients (25.2% female and 74.8% male) were enrolled. In a 5-year follow-up (adherence rate: 45.7%), the average survival was 9.4±10.9 months. *HER2* overexpression was evident in 24%. There was no statistically significant association found between *HER2* expression and primary tumor location (p-value=0.63), histopathological type (p-value=0.72), or tumor grade (p-value=0.051). Furthermore, no statistically significant links were apparent with tumor grade in either male or female groups as well as patients aged ≥60 and <60 years (all p-values >0.05). Moreover, no statistically significant association was detected between *HER2* expression status (p-value=0.88), sex (p-value=0.31), and age (p-value=0.055) with patient survival. **Conclusions:** No statistically meaningful association was found between all parameters examined and *HER2* expression status. Divergence of the results from earlier studies might be due to genetic variation. Thus, performing a meta-analysis on certain races might be helpful for clarification.

Keywords: *HER2*- stomach neoplasm- survival- cohort studies- Iran

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Introduction

Nowadays, cancer is considered a life threatening issue by being the second cause of death globally. Also, due to the different complications caused by disease and/or treatment, a wide range of morbidities is observed (Jemal et al., 2011). The gastrointestinal tract is one the most cancer-prone organs of the human body as gastric cancers (GCs) are considered the fourth most prevalent cancer worldwide and the second major cause of death in developing countries (Kamangar et al., 2006; Ferlay et al., 2010).

So far, many factors such as oncogenes have been known to directly associate with the risk of cancer as well as other environmental inducers. Human epidermal growth factor receptor2 (*HER2*) or CD340 is an encoded proto-oncogene by ERBB2 located on chromosome 17 (17q12-q21). This proto-oncogene belongs to HER family (HER1to HER4) with a tyrosine kinas activity. *HER2* is not tissue- specific and rather expressed in various tissues such as breast, gastrointestinal tract, heart, ovary, and kidney. According to the data, *HER2* plays an important role in cell cycle. This proto-oncogene promotes cellular proliferation and inhibits apoptosis. Considering the mentioned role, both proliferation induction and apoptosis inhibition may lead to cancer in an otherwise suitable situation (Coussens et al., 1985; Yamamoto et al., 1986; van der Geer et al., 1994; Neve et al., 2001; Olayioye, 2001; Ménard et al., 2003).

The first malignancy known to be associated with *HER2* over expression was breast cancer (Slamon et al., 1987). According to the previous studies, in 10-34% of patients diagnosed with invasive breast cancer, *HER2* overexpression and/or amplification was detected. Today,

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for breast cancer, positive *HER2* status has been considered as a negative predictor of response to chemotherapy, outcome of disease, and patients' survival (Kaptain et al., 2001). In 1986, using immunohistochemistry (IHC) a relation between GC and *HER2* overexpression was described for the first time (Sakai et al., 1986).

Nowadays, surgery is no longer considered the main management process for GC, as second-line therapies such as combination of chemotherapy and radiotherapy may also be helpful for patients with non-adequate response to the surgery or those who are not suitable candidates for surgery as the first line treatment (Cunningham et al., 2006).

So, the aim of this study was to evaluate the possible associations between *HER2* expression with prognostic, diagnostic, and demographic factors in patients with GC.

Materials and Methods

Patients and methods

Study design and ethics

This cohort study was conducted on samples collected between April 2008 and September 2013 in Vali-e-Asr Hospital (Zanjan, Iran). All individuals whom their samples were used in this study had given a signed consent form freely for any molecular and demographic studies (then and future). The inclusion criteria was defined as certain diagnosis of GC. Also, patients who had received any treatment such as chemotherapy, radiotherapy, and biological medication (monoclonal antibodies) before the sampling were excluded from the study. The study was approved by Hospital Medical Ethics Committee. All authors followed the 1964 Helsinki declaration and its later amendments.

Sampling and data collection

All the demographic information such as age and sex were extracted from the patients' files as well as tumors' anatomical location, and grade according to the WHO classification (Flejou, 2011). Histopathologic types of tumors were determined by reviewing slides of patients by an expert pathologist in pathology department of Vali-e-Asr hospital according to the Lauren's classification (Lauren, 1965).

All the samples were collected through two methods: endoscopic biopsy and tumor excision by curative or palliative surgery. After formalin fixing and paraffin embedding, all tissue samples were archived in Pathology Department of Vali-e-Asr Hospital.

Samples were prepared for immunohistochemistry (IHC) method according to the Canene-Adams's method in order to evaluate *HER2* expression. The ICH was performed by HercepTest kit (Dako, Glostrup, Denmark) according to the manufacture's guide. Finally, all samples were blindly evaluated for *HER2* expression by an experienced expert pathologist. The pathologist scored *HER2* expression according to the scoring criteria introduced by Hofmann et al., (2008) In this criteria, scores of 0 and 1 were considered as negative, score 2 was considered as intermediate, and score 3 was considered strong positive *HER2*.

Statistical analysis

For data analysis, IBMSPSS version 21.0 (SPSS, Chicago, IL, USA) was used. For analyzing the survival rate in patients (with and without *HER2* over expression), the Kaplan-Meier method was applied. Chi square method was used for evaluation of any possible statistically difference between two groups. A P-value of less than 0.05 was considered statistically significant.

Results

In this study, 210 patients with the average age of 68.9±12.16 years (ranged: 35-89) were included after meeting both inclusion and exclusion criteria. Among this number, 53 were female (25.2%) and the other 157 were male (74.8%). Although the race of participants was unclear, according to the general population of city/province it seems that the majority were Azeri. Considering the Lauren's classification, histopathological evaluations showed that 58.1% (N=122) and 25.7% (N=54) of samples were intestinal and diffuse type, respectively, and the remaining 16.2% (N=34) were considered as other. Also, tumor location and grades in all 210 patients are shown in Table 1 and Table 2, respectively. In this study, a 5-year

Table 1. Frequency of Primary Anatomical Region of Gastric Cancer

Tumor Location	Number	%
Cardia	45	21
Fundus	3	1.4
Lesser curvature	52	24
Body and antrum	40	19
Pylorus+pre pylorus	25	11.9
Esophagus	1	0.5
Duodenum	2	1
Cardia + fundus	3	1.4
Cardia + LC	1	0.5
Cardia + body	2	1
Lesser curvature + antrum	5	2.4
Lesser curvature + pylorus	2	1
Fundus + body	4	1.9
Body + Pylorus	6	2.9
Fundus + lesser curvature + body	2	1
Unknown	17	8.1
Total	210	100

Table 2. Frequency of Tumor Grade

Grade	Number	%
1	48	22.9
2	67	31.9
3	88	41.9
1-2	2	1
2-3	3	1.4
Unknown	2	1
Total	210	100

Table 3. Tumor Primary Location According to the HER2 Expression Status

		Tumor Location				
		Cardia	Body	Pylorus and Pre-Pylorus	Other	Total
HER2	Negative over-expression	70% (14)	70% (28)	63.6% (7)	88.9% (8)	71.3% (57)
	Positive over expression	30% (6)	30% (12)	36.4% (4)	11.1% (1)	28.8% (23)
	Total	100% (20)	100% (40)	100% (11)	100% (9)	100% (80)

Table 4. Results of HER2 Expression Status According to the Tumor Grade in Two Different Sex Category

Sex	HER2 expression status	Tumor grade			Sum
		Grade 1	Grade 2	Grade 3	
Male	Negative	30 (75%)	33 (63.5%)	52 (82.5%)	115 (74.2%)
	Positive	10 (25%)	19 (36.5%)	11 (17.5%)	40 (25.8%)
	Sum	40 (100%)	52 (100%)	63 (100%)	155 (100%)
Female	Negative	7 (77.8%)	13 (76.5%)	23 (85.2%)	43 (81.1%)
	Positive	2 (22.2%)	4 (23.5%)	4 (14.8%)	10 (18.9%)
	Sum	9 (100%)	17 (100%)	27 (100%)	53 (100%)

follow-up was carried out which unfortunately only 45.7% (N=96) of patients/family members adhered to it. According to the follow-ups, the average survival of patients was 9.4±10.92 months (ranged: 1-57).

Following the IHC results, it was showed that the incidence rate of *HER2* overexpression was 24% (N=50). Also, the other 76 % (N=158) of cases which were negative and borderline for *HER2* expression were classified in negative *HER2* overexpression group (two cases were defined as missing data in this section). The distribution of the tumor's primary location according to the *HER2* expression status is illustrated in table 3. Also, there was no statistically significant association found between tumor grade and *HER2* expression status (p-value=0.051). Likewise, no statistically significant association was detected between *HER2* expression status and tumor grade in either man or woman groups (p-value for the man=0.06 and p-value for woman=0.74). The distribution of tumor grade according to the *HER2* expression status in two groups of male and female are provided in Table 4 as well as distribution of histopathological types of tumor (Table 5). Moreover, the patients were categorized into two age groups with the cutoff point of 60 years (< 60 and 60≤ years old). For neither of the mentioned groups a statistically significant association was found between tumor grade and *HER2* expression status (p-value for the <60 years old group= 0.94 and p-value for 60 ≤ years old=0.17). The distribution of tumor grade according to the *HER2* expression status in the two mentioned age groups is gathered in table 6. Moreover, the findings showed no statistically significant association between *HER2* status and anatomical location of the tumor (p-value=0.63) as well as the histopathological type of the tumor (p-value=0.72).

The results of Kaplan-Meier analysis for *HER2* expression status, sex, and the mentioned age category are shown in Figure 1. According to the analysis, it was showed that there was no statistically significant association between each of *HER2* expression status (p-value=0.88), sex (p-value=0.31), and age (p-value=0.055) with the survival of patients.

Table 5. Tumor Histopathological Type According to the HER2 Expression Status

		Tumor histopathology			
		Intestinal	Diffuse	Other	Total
HER2	Negative over-expression	73.8% (91)	75.9% (41)	82.3% (28)	76.2% (160)
	Positive over expression	26.2% (31)	24.1% (13)	17.7% (6)	23.8% (50)
	Total	100% (122)	100% (54)	100% (34)	100% (210)

Table 6. Results of HER2 Expression Status According to the Tumor Grade in Two Different Age Category

Age (years)	HER2 expression status	Tumor grade			Sum
		Grade 1	Grade 2	Grade 3	
30-59	Negative	6 (66.7%)	10 (71.4%)	11 (73.3%)	27 (71.1%)
	Positive	3 (33.3%)	4 (28.6%)	4 (26.7%)	11 (28.9%)
	Sum	9 (100%)	14 (100%)	15 (100%)	38 (100%)
≥60	Negative	28 (77.8%)	35 (70%)	59 (84.3%)	122 (78.2%)
	Positive	8 (22.2%)	15 (30%)	11 (15.7%)	34 (21.8%)
	Sum	36 (100%)	50 (100%)	70 (100%)	156 (100%)

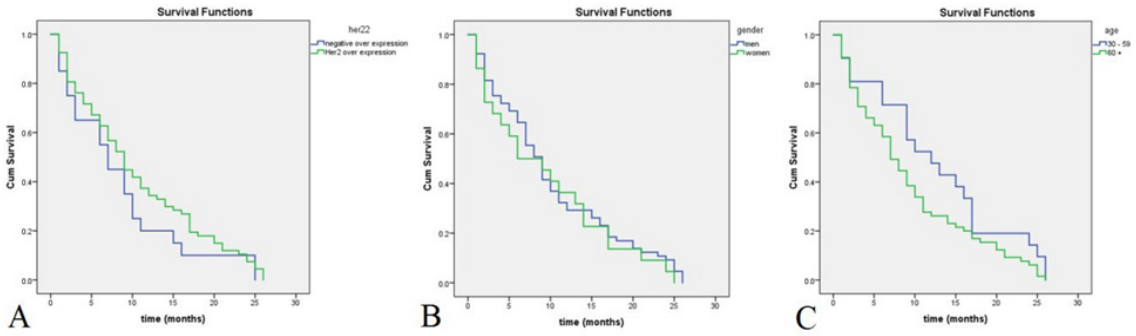

Figure 1. Results of Survival Analysis in 3 Different Categories. A, HER positive and negative over-expression; B, Sex; C, Age.

Discussion

Today, cancer is one the most important challenging issues faced in medical sciences. Among the wide range of different types of cancers, GC has become a hot topic due to its notable prevalence and poor prognosis (especially in advanced stages) with approximate 990,000 new cases each year leading to death of around 738,000 of mentioned number. The age standardized ration of GC is different in each geographical region ranging from 65.9 (in South Korea) to 3.3 (in Egypt) evaluated in male population (Karimi et al., 2014). As it has been reported, GC has the highest mortality rate (15.5%) among all the cancers and is the second prevalent (11.4%) of them in Iran (Cancer, 2012).

It is very important for both clinicians and patients to be aware of prognosis of the disease especially in the case of cancer. *HER2* is an already affirmed prognostic factor in breast cancer associating with poor prognosis (Kaptain et al., 2001). As mentioned before, the aim of this study was to evaluate the possible relation of *HER2* expression with survival of patients diagnosed with GC. According to the results, no statistically significant relationship was found between overexpression of *HER2* with each of grades (different sex and age groups), anatomic region, histopathology of tumor, and patients' survival outcome. Thus, it seems that *HER2* is not a helpful prognostic factor for the mentioned variables. On the other hand, there are studies supporting the fact that some of these variables are associated with *HER2* overexpression (Kim et al., 2012). This divergence might be due to the geographical or genetic differences between the evaluated populations.

In a study by Park et al., (2006) (South Korea) on 182 patients with GC, *HER2* expression status was evaluated by chromogenic in situ hybridization (CISH) and fluorescent in situ hybridization (FISH). They showed that *HER2* expression was positive in 15.9% (N=29) of their patients by ICH and in 7 cases *HER2*/nue gene amplification was observed by FISH and CISH. They also demonstrated that the intestinal type of GC was associated with a statistically different expression (higher) of *HER2* in comparison to the diffuse type. Moreover, they asserted that the TNM stage of cancer, *HER2* expression, and age were only independently related to survival by multivariate analysis.

Another study from South Korea by Kim et al., (2012) 9% (N=10) has been performed on GC patients with *HER2* positive status affirmed by IHC. They found statistically significant differences between *HER2* positive and negative groups in variables of histopathological type (Lauren classification) and differentiation status but not in primary anatomical site, age, and sex.

The other study by Halon et al., (2012) (Poland) on 78 cases diagnosed with GC showed that *HER2* was positive in 29.5% (N=23) of their studied population. It was also demonstrated that only TNM stage and patients' age were crucial negative prognostic factors. Also, they did not find any relation between *HER2* expression status and patients' survival.

To be more specific in comparison of data with other studies, it is better to evaluate the same studies from Iran. Raziei et al., (2007) studied 100 cases of GC from northeastern Iran for *HER2* expression using IHC. Among their patients, 26% (N=26) were positive for *HER2* expression; which showed a statistically significant relation with histopathological type (Lauren classification) and differentiation status but not age, sex, TNM stage, primary anatomical region of tumor, and metastasis. However, they did not evaluate patients' survival in their study.

In another study by Ansari et.al., (2011) (Iran) on 100 patients with GC, *HER2* expression was evaluated by IHC. They showed that only 7% (N=7) of their patients were *HER2* positive (3+ membranous *HER2* activity). However, they found no statistically significant difference between *HER2* expression status and differentiation status as well as survival duration.

Basi et al., (2015) (Iran) have also performed a study on 115 patients with GC using IHC. According to their results, *HER2* overexpression was detected in 11.3% of patients (N=13). Their results didn't show any statistically significant relation between *HER2* overexpression and different variables such as tumor histopathology (Lauren classification), TNM stage, and primary anatomical region of the tumor.

Considering all the mentioned results from different countries and even the studies from Iran, it is clear that the mentioned prognostic variables are still controversial, even two of the discussed studies from the same country of South Korea reached different results (Park et al., 2006; Kim et al., 2012). Although the results of mentioned studies from Iran were not much different from the current study, there is still some controversial issues. The main reason behind this issue could be the genetic differences in

various races focused in a country border. Thus, it seems that performing the same evaluation but in a specific race may be more accurate. The other possible affecting variables might be the differences in methodology of investigating *HER2* expression which influences the incidence of *HER2* expression in the studied population. It seems that it is better to use other complementary methods for detection of *HER2* overexpression unfortunately not performed in this or some other mentioned studies.

It is very important to assess the possible relation between *HER2* expression and prognosis and survival. If this biomarker is known to be involved in the prognosis and survival of patients with GC, the use of biological agents as a therapeutic method could be beneficial. Trastuzumab is a monoclonal antibody against *HER2* which has been successfully used for treatment of *HER2* positive breast cancer patients (Swain et al., 2015). In a multi-national phase 3 clinical trial on *HER2* positive gastric or gastro-esophageal junction cancer, it has been shown that the use of Trastuzumab in combination with chemotherapy statistically increases the median survival of patients in comparison to the group receiving chemotherapy alone (Bang et al., 2010).

Taken together, this study showed that there is no statistically difference between two groups of patients with and without *HER2* overexpression in variables such as survival, histopathological type of cancer (according to the Lauren classification) and primary anatomical region of tumor. Although, a very close (p-value=0.051) association between *HER2* expression and tumor grade was seen in the results. This association might not be statistically significant but it seems to be clinically important. Moreover, it was showed that there were differences in the results of current study with other studies especially on non-Iranian patients. Authors strongly suggest for future studies to focus on the race of patients accompanied by more exact evaluation of *HER2* expression status as well as its polymorphism. Moreover, it seems that due to the genetic variety of patients, it is better to perform a meta-analysis on the same race or at least with geographical restrictions.

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