**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

**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 treatment, a wide range of morbidities is observed (Jemal et al., 2011). The gastrointestinal tract is one of 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 (HER1 to 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,
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

| 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 |

| 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 |
HER2 Expression in Gastric Cancer

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.

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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%) |

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%) |

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.
Discussion

Today, cancer is one of 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 ratio 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/mue 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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