

HER2 Protein Expression in Gastric Carcinoma of Thai Patients at Phramongkutkloa Hospital

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ABSTRACT

Background: Gastric cancer (GC) and gastro-oesophageal junction cancer (GEJC) are major causes of morbidity and mortality worldwide. Human epidermal growth factor receptor 2 (HER2)-positivity is a significant negative prognostic factor for GC and a wide range of HER2-positivity rates (6-35%) have been reported. The international ToGA trial (trastuzumab with chemotherapy in HER2-positive advanced GC) is the first large phase III trial to provide prospective information on the incidence of HER2 positivity in advanced GC.

Objective: To assess the percentage of HER2 positivity in gastric carcinoma by immunohistochemistry (IHC) for protein overexpression and the correlation with histological findings.

Methods: Formalin-fixed, paraffin-embedded tumor samples were collected from GC patients in Phramongkutkloa hospital. The HER2 status of GC samples was determined using previously validated HER2-detection methods: immunohistochemistry (IHC) by primary immunoreagent 4B5 (PATHWAY_HER-2 rabbit monoclonal antibody) using the Ventana automated slide staining IHC system. Samples identified as IHC 3+ were defined as HER2 positive. We analyzed these HER2 positivity findings in relation to the status of tumor and to the histological subtype.

Result: Tumors from 68 patients were centrally tested for HER2 status: 18 of 68 cases (26.5%) were HER2. Tumor types (Lauren's classification) are 36 cases of intestinal type, 22 cases of diffuse type, and 10 cases of mixed type. Tumor grades are 8, 24, and 36 cases of well, moderate, and poorly differentiated tumors respectively. Age, sex and pathological subtype are not significant affected HER2 status in both univariate and multivariate analysis. Only tumor grading has shown correlation with HER2 overexpression.

Conclusion: The prevalence of HER2-positive in gastric cancer from this study was similar to other studies in Western countries and had highest percentage of intestinal sub-type in HER2-positive. Interestingly, we found that high prevalence of well-differentiated in HER2-positive which has been postulated that there are other unknown factors which related clinical outcome beside HER2 over-expression.

Key words : Human epidermal growth factor receptor, HER2, gastric cancer

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INTRODUCTION

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide⁽¹⁾ and it remains difficult to cure, primarily because most patients present with advanced disease. Even patients who present in the most favorable condition and who undergo curative surgical resection often die of recurrent disease.

Most gastric cancer patients are diagnosed when the tumor is at an unresectable and advanced stage which systemic chemotherapy is the main treatment option, however, survival outcome remain poor, usually less than 30% at 5 years⁽²⁾. So the new and less toxic treatments are urgently needed. A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies which interfere with the signaling cascades involved in cell differentiation, proliferation, and survival. One of them is the human epidermal growth factor receptor 2 (HER2), a 185-kD glycoprotein with tyrosine kinase activity. HER2 overexpression of breast cancers is a key feature of the pathobiology of the disease and is associated with poorer prognosis⁽³⁾. Many studies suggest the HER2 positively is an independent predictor of breast cancer recurrence and mortality^(4,5).

Trastuzumab, a monoclonal antibody that targets the extracellular domain of HER2, has shown survival benefits when given with chemotherapy (CT) in patients with HER2-positive early and metastatic breast cancer^(6,7). This efficacy has led to investigate its anti-tumor activity in patients with HER2-positive other cancers, including gastric adenocarcinomas⁽⁸⁻¹³⁾.

Consequently, HER2 status assessment in gastric carcinoma were reported around 16 - 23% positive cases (protein overexpression) and it has been correlated with poor prognosis and more disease aggressiveness.

The outcome of treatment from phase III ToGA (Trastuzumab for Gastric Cancer) study comparing trastuzumab in combination with standard chemotherapy versus standard chemotherapy alone for the treatment of HER2-positive advanced gastric cancer has been shown survival benefit, overall survival of about 13.5 months in the treatment group compared with 11.1 months in the control group. The benefit was greatest in patients with higher expression of HER2⁽¹⁴⁾.

In Thailand, the incidence of gastric cancer is approximately 3.9/100,000 per year^(15,16) but lacking of supported data to determine incidence of HER2 overexpression among gastric cancer patients. The primary end point of this study is to assess the percentage of HER2 positivity in Thai gastric cancer patients using immunohistochemistry (IHC) technique. The secondary end point is finding the correlation of HER2 status with patient characteristic, histological subtype and grading.

PATIENTS AND METHODS

Patients

Patients with histopathological report confirmed diagnosis of adenocarcinoma gastric cancer in any stage during 1 January 2005 - 31 December 2011 in Phramongkutklo hospital were eligible. There were 68 of 96 (70%) paraffin-embedded tumor samples that available to evaluated HER2 overexpression by IHC. Medical records of all patients were reviewed and demographic data were recorded including age, sex, histopathology subtype and grading. The study protocol was approved by Ethics Committees.

HER2 immunohistochemical stain

Paraffin-embedded tumor samples were reviewed by one pathologist who classified histologically according to the WHO histological classification of adenocarcinoma gastric tumors and evaluate for include tumor histologic type, subtype, grade and percentage of the immunoreactivity concerning pattern of staining and intensity.

HER2 status by immunohistochemistry (IHC) expression was evaluated by primary immunoreagent 4B5 (PATHWAY_HER-2 rabbit monoclonal antibody) using the Ventana automated slide staining IHC system. For the determination of HER-2/neu protein overexpression, only the membrane staining pattern and intensity of invasive tumor cells were scored.

Immunoreaction was determined as weakly positive (2+) if weak to moderate complete membrane staining in more than 10% of the tumor cells, strong positive (3+) if a strong complete membrane staining in more than 10% of the tumor cells. For small tissue by biopsy, if a complete membrane staining more than 5% of the tumor cells was defines as positive. Samples identified as IHC 3+ was defined as HER2 positive and

IHC 2+ was defines as equivocal that should be further investigate. All other staining patterns were interpreted as negative (0 or 1+).

Statistical method

The primary end point is prevalence of HER2-positive gastric cancer and the secondary end point is relationship of HER2 overexpression, tumor subtype and grading. Patients were categorized according to the HER2 status. Patient characteristics were compared between groups with X^2 test and patients' mean age was compared with ANOVA test. The statistical analyses were performed by using STATA software version 10. All p -value < 0.05 was considered statistically significant.

RESULTS

Ninety-six gastric cancer patients were enrolled in this study. There were 68 (70%) paraffin-embedded tumor samples that available to evaluated HER2 overexpression by IHC. Demographic data of all patients were listed in Table 1. Mean age was 62.68 ± 15.37 years.

There were 34 men (50%) and 34 women (50%) equally. Only 4 cases were esophago-gastric junction tumor and the others were solely gastric tumor. Tumor types (according to Lauren's classification) were 36 cases of intestinal type, 22 cases of diffuse type, and 10 cases of mixed type. Tumor grades are 8, 24, and 36 cases of well, moderate, and poorly differentiated tumors respectively. The IHC results of HER2 reveal 18 cases of complete membrane stain (more than 10% of tumor cells) with intense density, 12 cases of complete membrane stain (more than 10% of tumor cells) with weak density, 16 cases of partial membrane stain and 22 cases of negative stain neither membranous nor cytoplasmic stains.

Eighteen patients (26.5%) are HER2-positive gastric cancer, while 12 patients (17.6%) are equivocal and 38 patients (55.9%) are HER2-negative. Mean age was 58.89 ± 18.50 years in HER2-positive group, 64.67 ± 17.83 years in equivocal group and 63.84 ± 13.52 in HER2-negative group. There was no difference among these 3 groups.

Histopathological features were assessed for association with HER2 status. The HER2 status that significantly associated with tumor grading, while histological subtypes were not. The results were summa-

Table 1. Patient demographic and clinical characteristics.

Characteristic data	N = 68
Sex (%)	
Male	34 (50.0%)
Female	34 (50.0%)
Age (mean \pm SD, yrs.)	62.68 \pm 15.37
Type (%)	
Intestinal	36 (52.9%)
Diffuse	22 (32.4%)
Mixed	10 (14.7%)
Grade (%)	
Well differentiated	8 (11.8%)
Moderate differentiated	24 (35.3%)
Poorly differentiated	36 (52.9%)

rized in Table 2.

For HER2 positive GC, 12 of 18 patients (66.7%) are intestinal type, 2 of 18 (11.1%) is diffuse type and 4 of 18 (22.2%) are mixed type. Of 38 patients with HER2-negative, 18 (47.4%) are intestinal type, 16 (42.1%) are diffuse type and 4 (10.5%) are mixed while 12 of equivocal group are 6 (50%), 4 (33.3%), 2 (16.7%) of intestinal, diffuse and mixed type tumor respectively. In HER2 positive group, intestinal type was higher than the others, but this was not statistically different in sub-type of gastric cancer ($p=0.584$) as shown in Figure 1.

The data showed that eighteen patients of HER2 positive group, six patients (33.3%) are well differentiated, eight patients (44.4%) are moderately differentiated and four patients (22.2%) are poorly differentiated tumor. There were 38 patients with HER2 negative, are moderately differentiated (10 patients; 26.3%) and poorly differentiated (28 patients, 73.7%) but none of well differentiated. In equivocal group, twelve patients which is only two (16.7%) has well differentiated, six patients (50%) are moderately differentiated and four patients (33.3%) are poorly differentiated. There was statistically significance different ($p=0.033$) in grading histology for HER2 status as shown in Figure 2.

Subgroup analysis was shown that well differentiated tumor was significantly higher than the other subtypes in HER2 positive group ($p=0.035$) as shown in Figure 3. In the other hand, poorly differentiated was highest prevalence for HER2 negative group

Table 2. Patient demographic and histological features by HER2 status.

Characteristic data	HER2 negative (N = 38)	Equivocal (N = 12)	HER2 positive (N = 18)	p-value
Age (years)				0.535
Mean ± SD	63.84 ± 13.52	64.67 ± 17.83	58.89 ± 18.50	
Sex				0.698
Male	22 (57.9%)	4 (33.3%)	8 (44.4%)	
Female	16 (42.1%)	8 (66.7%)	10 (55.6%)	
Subtype				0.584
Intestinal	18 (47.4%)	6 (50.0%)	12 (66.7%)	
Diffuse	16 (42.1%)	4 (33.3%)	2 (11.1%)	
Mixed	4 (10.5%)	2 (16.7%)	4 (22.2%)	
Grade				0.033
Well differentiated	0 (0%)	2 (16.7%)	6 (33.3%)	
Moderate differentiated	10 (26.3%)	6 (50.0%)	8 (44.4%)	
Poorly differentiated	28 (73.7%)	4 (33.3%)	4 (22.2%)	

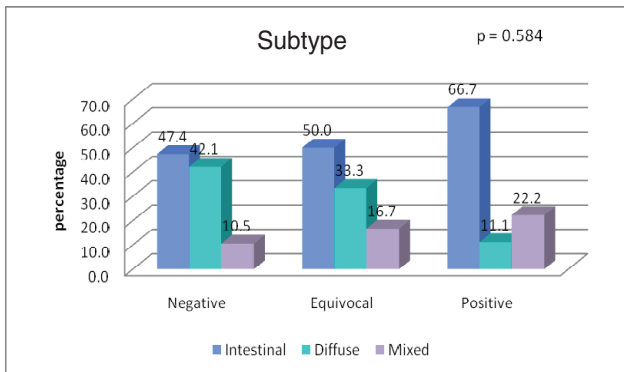


Figure 1. The relation between tumor subtype and HER2 status.

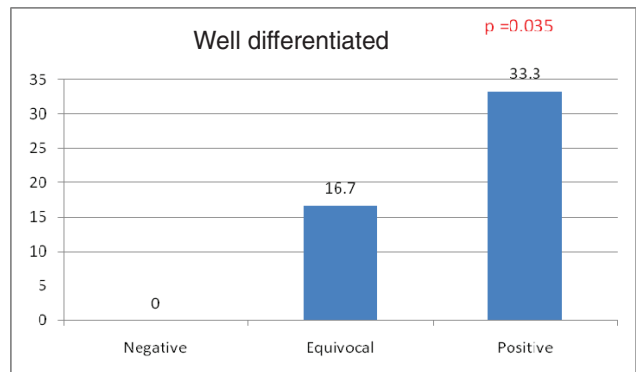


Figure 3. The percentage of tumor grading (well-differentiated) by HER2 status.

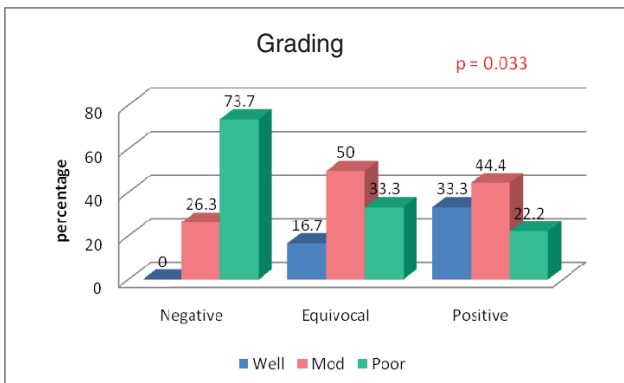


Figure 2. The relation between tumor grading and HER2 status.

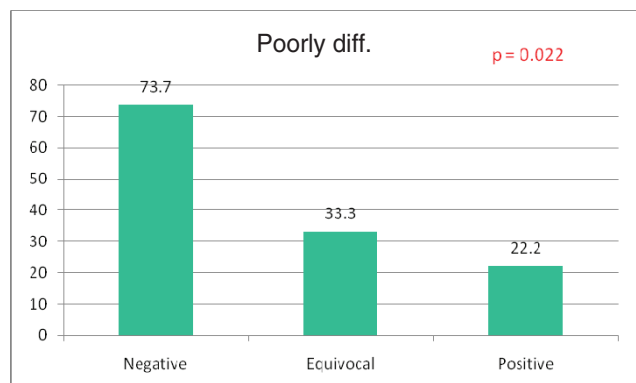


Figure 4. The percentage of tumor grading (poorly-differentiated) by HER2 status.

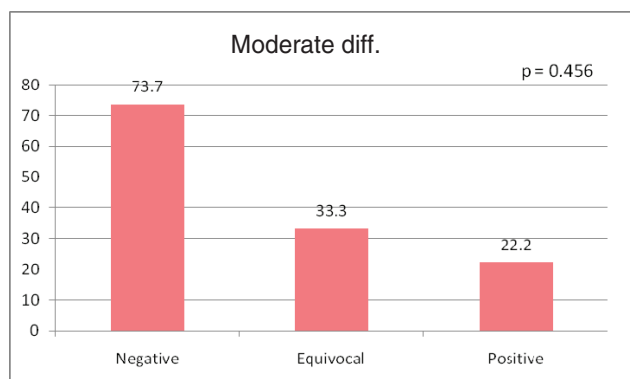


Figure 5. The percentage of tumor grading (moderate-differentiated) by HER2 status.

($p=0.022$). Additionally, moderate differentiated group was not different in HER2 status ($p=0.456$) as shown in Figure 4 and 5.

DISCUSSION

The TNM stage is the most important prognostic factor for gastric cancer. However, the prognosis varies among patients in the same stage. Therefore, additional classification parameters need to be defined in addition to the TNM and the classic pathologic characteristics of the tumor in order to better identify the biologic subsets of this disease such as biomarkers.

Over-expression of HER2 protein in gastric cancer, using immunohistochemistry (IHC), was first described in 1986, and it has been correlated to poor outcomes and a more aggressive disease. Until discovery of trastuzumab (Herceptin[®]), a monoclonal antibody against HER2, has shown survival benefits when given with chemotherapy (CT) in patients with HER2 positive both early and metastatic breast cancer which has been approved by global FDA for standard treatment.

According to the efficacy of trastuzumab in breast cancer patients has led to investigate its antitumor activity in patients with HER2-positive cancers, including gastric adenocarcinomas. The outcome of treatment from phase III ToGA study has been shown survival benefit. The median OS was significantly improved with trastuzumab add on standard chemotherapy (H+CT) compared to standard chemotherapy alone (CT) (13.5 versus 11.1 months, respectively; $p=0.0048$, HR 0.74; 95% CI 0.60, 0.91). Overall risk reduction (ORR) was 47.3% in the H+CT arm and 34.5% in the CT arm ($p=0.0017$)⁽³⁾.

In Western countries, some series reported a 9%-38% of HER2-positive. More recent studies, the ToGA is a multicenter, international trial to be conducted at 130 centers in Europe, Russia, Japan, Korea, China, Taiwan, Australia, Central and South America, South Africa, India, and Turkey, which centrally determined HER2 over-expression by IHC and FISH, 341 (22%) of 1527 tumors were HER2 positive.

From this study, thirty-four patients with gastric cancer whom had adequate in medical records and specimens were registered at Pathology unit, Phramongkutkloa hospital during the 7-years period. The prevalence of HER-2 positive in our result (26.5%) was within the same range as found in a FISH-based study recently reported by ToGA trial, who found HER2 to be amplified in 22% of the cases.

Tanner et al.⁽¹⁷⁾ observed HER2 amplification by chromogenic in situ hybridization (CISH) in 12% of 131 gastric adenocarcinomas and in 24% of 100 GEJ tumors. HER2 amplification was strongly associated with the intestinal histologic type according to Lauren's classification (intestinal type 21.5%, diffuse type 2%, mixed/anaplastic type 5%; $p = 0.005$), but it was not associated with gender, age at diagnosis and clinical stage in any of the tumor groups studied. Our study observed a higher rate of HER2 over-expression in intestinal than diffuse and mixed type, although the different was not statistically significant which this study was effected on small sample size. Additionally, we found that mixed type also had stronger complete membrane staining in intestinal part than was diffuse part.

Tumor grading was significantly correlated with HER2 status; highly percentage of HER2 positive group was well-differentiated tumor. For moderately-differentiated tumor, which the part had strong complete membrane staining was the area that form gland (well differentiated).

In summary, these results had not only the same prevalence of HER2 positive in gastric cancer but also intestinal subtype with HER2 positive was similar to other previous studies in Western countries. Furthermore, we found that the correlation between grading of tumor and HER2 status, which had high prevalence of well-differentiated in HER2 positive gastric cancer. In fact, well differentiated tumor should be associated with the good outcome but this result was found high prevalence in HER2 positive. Therefore, it has been postulated that there are probably other unknown factors which related clinical outcome apart from HER2

overexpression. In Thai population, the further large sample size study is needed for the conclusion.

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