ENDOSCOPY. HISTOPATHOLOGICAL FINDINGS AND HER2 OVEREXPRESSION IN GASTRIC ADENOCARCINOMA

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Abstract:
Background and aim: HER2 is considered to have prognostic value in gastric cancer and to test for HER2 overexpression to help to select candidates for targeted therapy with trastuzumab. This study is aimed at the assessing endoscopic and histopathological characteristics of gastric adenocarcinoma and their relationship with HER2 overexpression. **Objectives and methods:** Biopsy samples from 64 gastric cancer patients were examined for HER2 status by immunohistochemical staining. **Results:** 7.8% of tumors were cardia tumors and 92.2% were non-cardia tumors. Using the Lauren classification, 42.2% were intestinal type and 57.8% were diffuse type. Using WHO classification, 43.8% were tubular adenocarcinoma, 7.8% were mucinous adenocarcinoma, 17.2% were signet-ring cell carcinoma, and 31.3% were undifferentiated carcinoma. 29.7% were well-differentiated, 18.8% were moderately-differentiated, and 51.6% were poorly-differentiated carcinoma. HER2 was positive in 23.5% of gastric carcinomas, 40% cardia tumors and 22% non-cardia tumors. HER2 positivity among polypoid, fungating, ulcerated, and infiltrative types were 30.8%, 37%, 6.3% and 0%, respectively. HER2 overexpression in intestinal type was higher than that in diffuse type (37% vs. 13.5%, $p = 0.04$). HER2 overexpression in tubular adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, and undifferentiated carcinoma was 35.7%, 9.1%, 20% and 15%, respectively. 36.8% of well-differentiated tumors, 33.3% moderately-differentiated tumors, and 12.1% of poorly-differentiated tumors were found to be HER2-positive. **Conclusions:** HER2 overexpression was found in 23.5% of gastric carcinoma and was associated with its endoscopic findings and histopathologic types.
Keywords: HER2, gastric cancer.

1. INTRODUCTION:

Gastric cancer is still the second most common cause of cancer death worldwide, with high morbidity and mortality with 15.068 - 16.114 suffers and 11327 - 12098 deaths each year in Vietnam [3]. Despite advances in diagnosis, surgery and chemotherapy, the prognosis of gastric carcinoma remains poor. So, more studies in prognosis and improvement of therapeutic outcome are required. HER2, a molecular marker of EGFR (Epidermal Growth Factor Receptor) family, is considered to have prognostic value in gastric cancer [12], [15]. Test for HER2 overexpression by immunohistochemistry (IHC) in patients with advanced gastric carcinoma helps to select

patients for targeted therapy with trastuzumab [1]. In Vietnam, there are not many studies about this yet.

This study is aimed at assessing the endoscopic and histopathological characteristics of gastric adenocarcinoma and their relationship with HER2 overexpression.

2. MATERIALS AND METHODS:

2.1. Patients:

Patients underwent consultations and endoscopy at Hue University Hospital and Hue Central Hospital, from 3/2010-5/2011, those suspected of gastric cancer and whose pathohistologic results on endoscopic biopsy specimen were gastric adenocarcinoma were included in this study.

2.1.1. Inclusion criteria:

Patients with a pathohistologic result of endoscopic biopsy specimen confirmed as gastric adenocarcinoma.

2.1.2. Exclusion criteria:

Patients with recurrent gastric carcinoma, secondary gastric carcinoma or combined with other cancers were excluded from the study.

2.2. Methods:

2.2.1. Design: cross-sectional study.

2.2.2. Techniques:

All patients with gastric lesions suspected of gastric cancer were observed, checked for lesion location (cardia, non-cardia), and classified according to Borrmann: type I: polypoid; type II: fungating; type III: ulcerative; type IV: infiltrative. Biopsy with strip technique was performed after observation and assessment. Biopsies should include samples from the base and the lesion rim in all quadrants. At least 6 samples were taken from each patient. The size of each specimen was 2-3mm. Biopsy samples were sent to Pathology Departments of Hue Univesity Hospital, Hue Central Hospital for HE staining and embedded in paraffin block, stored in room temperature. All were sent to cytology and pathology of Hanoi Cancer Hospital for HE test and IHC staining.

a) Pathohistological staining technique và pathohistological classification:

- Technique: Biopsy specimens were stained with HE and evaluated under optic microscopy.

- Pathohistological classification of gastric carcinoma:

+ Lauren classification: intestinal-type, diffuse-type and mixed-type.

+ WHO classification (2000): Papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, Adenosquamous carcinoma, squamous cell carcinoma, Undifferentiated carcinoma, and other types [11].

+ The degree of differentiation of gastric carcinoma was assessed according to World Health Organisation (2000): Well-differentiated carcinomas produce well-formed glandular structures that resemble the metaplastic intestinal epithelium. Poorly differentiated tumors are composed of irregular, poorly formed glands, or infiltrate as single cells or small cell clusters. Moderately differentiated cells demonstrate characteristics that are intermediate between well- and poorly differentiated lesions [11].

b) Immunohistochemistry:

- Technique: Immunohistochemistry for HER2 was performed by using the HercepTest kit (DakoCytomation, Denmark), according to the manufacturer's instructions.

- Immunohistochemical staining for HER2 was evaluated following the criteria recommended as follows [1]:

+ 0: No reactivity or no membranous reactivity in any tumor cell.

+ 1+: Tumor cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells stained.

+ 2+: Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained.

+ 3+: Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained.

Only scores of 2+ and 3+ staining levels were considered to be positive.

2.2.3. Statistical analysis:

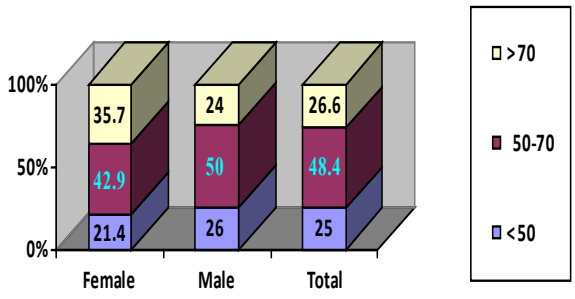
All statistics were performed two-sided, at a significant level of $P<0.05$, using the SPSS statistical software package for Windows (release 13.0; SPSS, Inc., Chicago, IL). Categorical data were analyzed using chi-square statistics.

3. RESULTS:

3.1. Characteristics of patients:

From 3/2010 to 5/2011, 64 patients were enrolled into the study. The yoxungest patient was 26 years of age, the oldest patient was 92 years of age; mean age was 60.2 +/- 13.8; median age was 58.5. 16 (25%) patients were less than 50, 31 (48.4%) patients were 50-70, 17 (26.6%) patients were more than 70. 50 (78.1%) patients were male, and 14 (21.9%) patients were female. The male/female ratio was 3.57/1. In both genders, patients aged 50-70 predominated. The emale and male rate of patients of 50-70 years old was 42.9% and 50%, respectively (Figure 1).

Figure 1: Characteristics of age and gender



3.2. Gross endoscopic and pathohistological characteristics:

Table 1: Gross endoscopic and pathohistological charateristics

	Total	Percentage
Tumor location		
Cardia	5	7.8
Non-Cardiac,	59	92.2
Fundus, Body	13	20.3
Lesser curvature	17	26.6
Antrum, pylorus	29	45.3
Borrmann classification		
Polypoid	13	20.3
Fulgating	27	42.2
Ulcerative	16	25.0
Infiltrative	8	12.5
Lauren classification		
Intestinal	27	42.2
Diffuse	37	57.8
WHO classification		
Tubular type	28	43.8
Signet ring cell type	11	17.2
Mucinous type	5	7.8
Undifferentiated type	20	31.3
Differentiation degree		
Well	19	29.7
Moderate	12	18.8
Poor	33	51.6
Total	64	100

Remarks: Non-cardiac gastric carcinoma predominates (92%). Among them, gastric carcinoma at antrum, pylorus was highest, followed by gastric carcinoma at lesser curvature. Fungating-type gastric carcinoma was highest, followed by polypoid type, ulcerative type (equivalent rate) and infiltrative type carcinoma was the least. According to the Lauren classification, diffuse-type was more common than intestinal-type. According to WHO classification, tubular type gastric carcinoma was highest (43.8%) followed by undifferentiated type (31.3%), signet ring cell type (17.2%), and mucinous type (7.8%). For degree of differentiation, poorly-differentiated type was highest (51.6%), followed by well-

differentiated type (29.7%) and moderately-differentiated type (18.8%).

3.3. HER2 overexpression and relationship between HER2 overexpression and tumor location, endoscopic, pathohistological characteristics of gastric carcinoma

3.3.1. HER2 overexpression in gastric carcinoma

Among 64 patients with gastric carcinoma, 9 patients were classified as score of 1+ HER2 overexpression, 9 patients were classified as score 2+ HER2 overexpression and 6 patients were classified as score 3+ HER2 overexpression (Table 2).

Table 2: HER2 overexpression in gastric cancer

HER2 overexpression	Number	Percentage (%)	HER2 positive
0	40	62.5	
1+	9	14.1	
2+	9	14.1	**23.5%**
3+	6	9.4	
Total	64	100	

Remark: 15 (23.5%) patients were classified as positive HER2 overexpression.

3.3.2. The relationship between HER2 overexpression and tumor location, and gross charateristics of gastric carcinoma:

Table 3: HER2 overexpression according to tumor location, gross characteristics, pathohistological classification of gastric carcinoma.

	Number n	HER2 Overexpression	Percentage (%)	p
Location				
Cardia	5	2	40	0.58
Non-Cardiac	59	13	22	
Borrmann				
Polypoid	13	4	30.8	
Fulgating	27	10	37	
Ulcerative	16	1	6.3	0.04
Infiltrative	8	0	0	
Total	64	15	23.5	

Remark: Cardiac tumors had more HER2 overexpression than noncardiac tumors (40% vs 22.2%). The difference, however, was statistically unsignificant. According to Borrmann classification, positive HER2 overexpression rate of polypoid type tumors, fungating type tumors and ulcerative type tumors was 4/12 (30.8%), 4/14 (37%), 1/8 (6.3%) respectively. Infiltrative type tumors did not overexpress HER2. The difference was statistically significant (p = 0.04).

3.3.3. The relationship between HER2 overexpression and pathohistological characteristics in gastric carcinoma

According to Lauren classification, intestinal-type cancers showed HER2 overexpression more than diffuse-type cancers (37.5% vs 13.5%, p = 0.04).

According to WHO pathohistological classification, HER2 overexpression of tubular type 35.7%, mucinous type 9.1%, signet ring cells type 20.0%, and undifferentiated type 15.0%. The difference was statistically insignificant (p = 0.22).

According to differentiation degree, HER2 overexpression of well-differentiated tumors, moderately-differentiated tumors and poorly-differentiated tumors were 36.8%, 33.3%, 12.1%, respectively. The difference was statistically insignificant.

Table 4: HER2 overexpression according to tumor location, endoscopic characteristics, and Lauren, WHO and differentiation degree classification

	Number n	HER2 overexpression	Percentage (%)	p
Lauren Classification				
Intestinal	27	10	37.0	0.04
Diffuse	37	5	13.5	
WHO classification				
Tubular type	28	10	35.7	
Signet ring cell type	11	1	9.1	0.22
Mucinous type	5	1	20.0	
Undifferentiated type	20	3	15.0	
Differentiation degree				
Well	19	7	36.8	0.09
Moderate	12	4	33.3	
Poor	33	4	12.1	
Total	64	15	23.5	

4. DISCUSSION

4.1. Characteristics of patients:

4.1.1. Age

Most of patients with gastric carcinoma in our study were concentrated in patients older than 50 (75% patients), especially patients of 51-70 years of age (approximately 50%). Age distribution was not different between gender. Mean age was 60.2. This rate was suitable for age characteristics of patients with gastric carcinoma in domestic and foreign studies [7], [8], [10].

4.1.2. Gender:

In domestic and foreign studies, the male/female rate was in the range of 1.82/1-2.8/1 [7], [8], [10], [14]. In our study, the male/female rate was 3.57/1, higher than the rate of the study of Nguyen Lam Hoa (2.5/1) [7], Nguyen Ngoc Hung (2.125/1) [8]; Raziee (2.8) [10]; T.V. Hợp (1.82/1) [14].

4.2. Endoscopic and pathohistological characteristics:

4.2.1. Gross characteristics

Non-cardiac gastric cancer was the most common (92.2%), gastric carcinoma and cardia was 7.8%. So, no significant increase in cardiac gastric carcinoma was seen. Among noncardiac gastric carcinoma, cancers at the antrum and pylorus were highest, followed by cancers at the lesser curvature. This rate corresponded to the rate in T. V. Hop study [14].

Fungating-type gastric carcinoma was highest (42.2%), the rate of polypoid type and ulcerative type is approximately similar, 20.3% and 25%, respectively; infiltrative type was least seen (12.5%). This rate was corresponding to the rate of domestic and foreign authors [4], [14]. N. V. Thanh, however, showed that ulcerative type gastric carcinoma was more frequently ~~seen~~ seen than other types (52.4%) [9].

4.2.2. Pathohistological

According to the Lauren classification, diffuse-type (57.8%) was more commonly seen than intestinal-type (42.2%). This was a little different from some other studies, which showed the intestinal-type was more commonly seen [8], [9]. The reasons for that was the number of undifferentiated type, signet ring cells type, and mucinous type patients was high. These were tumors which frequently showed pathohistological characteristics of diffuse-type [4].

According to the WHO classification, tubular-type gastric carcinoma was the most common (43.8%), followed by undifferentiated type (31.3%), signet ring cells type (17.2%) and mucinous type (7.8%). These rates were corresponding to the rates in the study of N. N. Hung et al [8].

Considering the degree of tumor differentiaion, the poorly-differentiated type was most commonly seen (51.6%), followed by the well-differentiated type (29.7%), and moderately- differentiated type (18.8%). This was a little different from some other studies. N. N. Hung showed the well-differentiated type was most commonly seen, followed by the moderately-differentiated type and poorly-differentiated type [8].

4.3. HER2 overexpression in gastric carcinoma:

In this study, we assessed HER2 overexpression of endoscopic biopsy samples by IHC. This technique is concordant with technique assessing HER2 amplification by fluorescent in situ hybridization (FISH) [16]. In our study, the HER2 overexpression rate was 23.5%, similar to the results of Yano in Japan (23%) [16], and the ToGA study (22%) [6] but higher than the result of N. V. Thanh [9]. These patients with positive HER2 overexpression were candidates who can be considered to have benefits from target therapy with transtuzumab [1].

4.4. Relationship between HER2 overexpression and tumor location, endoscopic, pathohistological characteristics of gastric carcinoma:

4.4.1. Relationship between HER2 overexpression and tumor location

Etiological and pathohistological characteristics of cardiac gastric cancers commonly different from those of non-cardiac gastric cancers. Cardiac gastric carcinoma showed HER2 overexpression more frequently than noncardiac tumors [2], [6], [12]. Our study also demonstrated HER2 overexpression of cardiac tumors (40%) was higher than non-cardiac tumors (22%). This result was corresponding to Gravalos [2], Tanner [12], and ToGA study [6] (Table 5). However, possibly due to the small sample, only 5 patients hadcardiac cancer, this difference was statistically unsignificant ($p > 0.05$).

4.4.2. The relationship between HER2 overexpression and gross characteristics in endoscopy

Gross characteristics according to the Borrmann classification was associated with pathohistological characteristics of tumors and had prognostic value. In this study, we revealed that gross characteristics were associated with HER2 overexpression: HER2 overexpression was different between lesion types at endoscopy, for example, 30.8% of polypoid type showed positive HER2 overexpresion, 37% of fungating type showed positive HER2 overexpresion, and 6.3% of ulcerative type showed positive HER2 overexpresion. Patients with gastric carcinoma infiltrative type did not show HER2 overexpression. This difference was statistically significant ($p < 0.05$).

4.4.3. The relationship between HER2 overexpression with Lauren histologic type

HER2 overexpressed in 25% intestinal-type cancers, which were higher than

diffuse-type tumors with only 20% HER2 overexpression ($p<0.05$). A lot of authors demonstrated similar results. Lemoine found that HER2 overexpression in patients with intestinal-type was higher than diffuse-type (53% vs 8%) [5]. In the more recent studies of Gravalos [2], Raziee [10], and Tanner [12], a strong association between HER2 overexpression and gastric carcinoma intestinal-type was confirmed (Table 5). Reasons for selective HER2 overexpression in intestinal-type gastric carcinoma were complicated and require further study because not all the intestinal-type cancers overexpressed HER2. Furthermore, intestinal-type gastric carcinoma was the cancer type with more favorable prognosis than the diffuse-type, while HER2 overexpression frequently related to poor prognosis. So, there must be a lot of factors influencing HER2 overexpression in patients with gastric carcinoma.

Table 5: HER2 Overexpression by Lauren histologic classification and tumor location.

Authors	n	Histologic type				Location			Method
		intestinal-type (%)	diffuse-type (%)	mixed (%)	P	Cardia (%)	Non-Cardiac (%)	P	
Gravalos et al. [2]	166	16	7	14	0.27	25	9.5	0.01	IHC, FISH
Lordick et al. [6]	1527	34	6	20	-	32	18	-	IHC, FISH
Tanner et al. [12]	231	21.5	2	5	0.005	24	12	-	CISH
Our study	64	37	13.5	0	0.04	40	22	0.58	IHC

4.4.4. The relationship between HER2 overexpression with WHO pathohistological classification and differentiation degree

According to this pathohistological classification, among 64 patients HER2 overexpression of tubular type gastric carcinoma was highest (35.7%), followed by signet ring cells type (20.0%), undifferentiated type (15%), and mucinous type (9.1%). These rates are similar to some other authors concerning the odds between pathohistological types, but the HER2 overexpresion rates in each group of our study were higher than the corresponding groups of other authors. Tateishi demonstrated that 9.2% of patients with tubular-type HER2 showing positive HER2 overexpression; only 4% of undifferentiated type showing positive HER2 overexpression [13]. Uchino found the rate of positive HER2 overexpression was 14% in the papillary and tubular adenocarcinoma, while the undifferentiated type or signet ring cells type showed very low rates of positive HER2 overexpresion (only 2%) [15].

According to WHO classification, the degree of gastric carcinoma was commonly associated with histologic type. Some studies showed that HER2 was more commonly overexpressed in well-differentiated tumors than other tumors. Raziee admitted HER2 overexpression in well-differentiated gastric carcinoma was 41% [10]. In patients with

undifferentiated type, Raziee found that 7% were positive HER2 overexpression [10] and Tateishi found HER2 positive 4% [13] while this rate in our study reached 12.1%. In our study, HER2 overexpression in well-differentiated tumors was 36.8%, higher than moderatelee-differentiated tumors (33.3%) and poorly-differentiated tumors (12.1%). However, the difference was statistically insignificant possibly due to a small sample.

5. CONCLUSION

- HER2 overexpression rate in patients with gastric adenocarcinoma was 23.5%. HER2 overexpression was associated with gross characteristics, and a histologic type of tumor.

Further studies are required to confirm the association between HER2 overexpression with endoscopic, pathohistological characteristics of gastric carcinoma and to recheck equivocal immunohistochemical cases with FISH.

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