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RESEARCH ARTICLE

Study of HER 2 expression in patients with Barrett's esophagus and esophageal adenocarcinoma

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ABSTRACT

Background: Barrett's esophagus (BE) is a premalignant lesion of the esophagus characterized by intestinal metaplasia. Once developed, it can progress to esophageal adenocarcinoma (EAC). Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase that regulates proliferation and differentiation. HER2 plays a role in the development of several types of cancer. However, the status of HER2 expression in EAC remains unclear. **Aim**: This study aims to evaluate the role of HER 2 expression as a potential marker for progression of Barrett's esophagus to esophageal adenocarcinoma and to examine its relationship with the clinicopathological features of the patients. **Material and Methods:** This study included 40 subjects; 30 patients with gastro-esophageal reflux disease (23 patients with BE and 7 patients with EAC) and 10 normal subjects as a control group. Complete blood count and fecal occult blood test were measured. Esophageal mucosa was evaluated by upper endoscopy and histopathological examination of gastro-esophageal junction was done. Scoring of HER2 expression was performed by immunohistochemical staining. **Results:** HER2 expression was significantly increased in patients with BE and those with EAC. HER2 expression was significantly higher in tumorous tissue than in BE. HER2 expression was positively correlated with the degree of dysplasia in BE patients and with TNM stage in EAC patients. **Conclusions:** HER2 expression correlated well with the degree of dysplasia in BE and its progression to EAC. HER2 expression is a potential biomarker for early detection of EAC. HER2 expression may have a role in esophageal carcinogenesis. Further well-designed prospective studies are required to prove this hypothesis.

Keywords: HER 2 Expression, Barrett's esophagus, esophageal adenocarcinoma, gastroesophageal reflux disease

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is the main risk factor for Barrett's esophagus and esophageal adenocarcinoma (EAC) with increased incidence by obesity, hiatus hernia, and tobacco (Illig *et al.,* 2013). Barrett's esophagus is a premalignant lesion of the lower esophagus that is characterized by intestinal metaplasia of the squamous epithelium. It can progress through varying grades of dysplasia to esophageal adenocarcinoma, which has a poor outcome unless diagnosed early. The pathophysiology of Barrett's metaplasia is incompletely understood but is related to chronic damage from gastric acid and bile reflux (Ong, Lao-Sirieix & Fitzgerald, 2010 and Bennett & Mashimo, 2014).

The GERD-Barrett-adenocarcinoma sequence currently lacks well-defined diagnostic, and prognostic biomarkers, providing an appropriate screening method for progression from Barrett's esophagus to adenocarcinoma (Booth & Thompson, 2012). The epidermal growth factor receptor (EGFR) family contains four members; EGFR, ErbB2/human epidermal growth factor receptor-2 (HER2), ErbB3/HER3, and ErbB4/HER4, that act as transmembrane receptor tyrosine kinases and have a well-defined function in the regulation of cellular processes that control cell growth, differentiation, and migration (Sergina &

Moasser, 2007). HER2 plays a role in the development and progression of several types of human cancer, including breast cancer, colorectal cancer, and esophageal carcinoma (Sergina & Moasser, 2007).

Reportedly, HER2 overexpression was detected in approximately 22% of advanced gastric cancers, (Gravalos & Jimeno, 2008) and targeting the extracellular domain of HER2 in these patients was associated with clinical benefit compared with chemotherapy alone in a phase III trial (Bang *et al.,* 2010). HER2 expression and its association with clinicopathologic features and clinical outcome in esophageal adenocarcinoma remain unclear (Tanaka *et al.,* 2012, Yoon *et al.,* 2012b and Nagaraja *et al.,* 2016). The correlation of HER2 expression with early neoplastic development in Barrett's esophagus has not been well explored, shows some discrepancies, (Rossi *et al.,* 2009 and Cronin *et al.,* 2011) and hence is the focus of this study.

The present work aimed to evaluate the role of HER 2 expression as a potential marker for the progression of Barrett's esophagus to esophageal adenocarcinoma and to examine its relationship with the clinicopathological features of the patients.

MATERIAL AND METHODS

This study was carried out on 40 subjects. 30 patients with gastroesophageal reflux disease divided into 23 patients with Barrett's esophagus and 7 patients with esophageal adenocarcinoma. Also, 10 healthy control subjects were involved. Patients were selected from those admitted or referred to the Endoscopy Unit of the Internal Medicine Department at Tanta University Hospitals. Written informed consent was obtained from all subjects.

Subjects were divided into the following groups: **Group I:** included 10 normal subjects as a control group (4 males and 6 females). **Group II:** included 23 patients with Barrett's esophagus (18 males and 5 females). The diagnosis of Barrett's esophagus was based on endoscopic findings (Figure 1) and confirmed by histopathological examination. **Group III:** included 7 patients with esophageal adenocarcinoma (5 males and 2 females). The diagnosis of esophageal adenocarcinoma was based on clinical, endoscopic findings and histopathological examination.

Exclusion criteria: All patients with other malignancies, other types of esophageal carcinoma and patients with previous chemo-radiotherapy were excluded from the study. All cases included in the study were subjected to the following: Full history taking: duration of GERD, history of smoking, previous drug intake and symptoms of rapid deterioration of general health. Complete clinical examination: General examination: pallor, palpitation, general weakness, etc. Abdominal examination: for liver, spleen, kidneys and any mass. Abdominal ultrasonography: Abdominal ultrasonography was done in the Internal Medicine Department for all cases. CT chest and CT abdomen: for detection of metastasis.

Laboratory investigations including complete blood count, liver function tests and fecal occult blood test. Occult blood in the stool was measured by a rapid chromatographic immunoassay technique. Upper gastrointestinal endoscopy was done using videoscope (GIF-Q 140 Olympus, Ltd. Japan) to investigate the studied groups. Special attention was made to the presence or absence of any finding of Barrett's esophagus and esophageal adenocarcinoma. Upper endoscopy with mucosal biopsy from gastro-esophageal junction was examined for: diagnosis of Barrett's esophagus and carcinoma, tumor grading and scoring the expression of HER 2 in tumorous and non-tumorous esophageal tissues by immunohistochemical staining.

During endoscopy the following landmarks were identified: Squamocolumnar junction, the esophago-gastric junction and lower esophageal sphincter. Grossly, Barrett's mucosa is usually represented by a well-defined area of salmon pink mucosa similar to adjacent gastric mucosa with irregular margins and may contain islands of the residual squamous white esophageal mucosa. The diagnosis of BE requires that the columnar epithelium extends above the gastro-esophageal junction (GEJ), and the presence of columnar metaplasia is confirmed in the esophageal biopsy. BE has

been divided into short segments (<3cm) and long segments (≥ 3 cm), depending on the length of the metaplastic epithelium. Four quadrant biopsies were taken by regular forceps and proceeding every 2 cm throughout the entire length of the columnar-lined esophagus. Specimen from adenocarcinoma patients were taken from the mass and adjacent mucosa.

Histopathological and immunohistochemical examinations

Histopathological examination: Paraffin blocks were made from the endoscopic biopsy previously fixed in 10% neutral formalin. Serial sections (5µm) were cut from each specimen. Sections were subjected to hematoxylin and eosin stain. Barrett`s esophagitis was diagnosed by the characteristic metaplastic changes of the esophagus, comprise proximal columnar epithelia with intestinal-type goblet cells (intestinal metaplasia), the junctional subtype with mucous secreting glands (cardiac metaplasia) and the gastric fundus subtype with parietal and cheif cells (fundic metaplasia). Dysplasia in Barrett's esophagitis was evaluated and classified according to Riddell's classification (Montgomery, Bronner & Goldblum, 2001) into: Negative dysplasia. Indefinite dysplasia: there is difficulty in distinguishing between dysplasia and regenerative change. Low-grade dysplasia: The atypical nuclei of dysplastic epithelium extend onto the mucosal surface so both the surface and the glands contain nuclei that are much larger and hyperchromatic than the nuclei in the unaffected epithelium. High-grade dysplasia: distortion of glandular architecture composed of branching and lateral budding of crypts, a villiform configuration of the mucosal surface, or intraglandular bridging of the epithelium to form a cribriform pattern. Esophageal adenocarcinoma was evaluated for grading (Matthew, Sreelakshmi & Sergei, 2012) into: Grade I:>95% of the tumor is gland forming. Grade II: shows 50-95% gland formation. Grade III: mostly solid with <50% gland formation.

Immunohistochemical examination (Mohamed, Samy & Ahmad, 2015): Immunohistochemical staining was performed on 3-5 mm sections from randomly selected paraffin blocks, using the Ultra Vision Detection System (Anti-Polyvalent, HRP/DAB ''Ready-to-Use'', Cat. # TP-015-HD, Lab Vision, USA).

The staining procedure was done as follows:

Deparaffinization and rehydration of sections: The slides were placed in a xylene bath overnight to remove the paraffin. The sections were then rehydrated by placing them in graded alcohol series, followed by rinsing with distilled water.

Blocking endogenous peroxidase: After deparaffinization and subsequent blockage of the endogenous peroxidase activity by incubation in 0.3% methanolic hydrogen peroxide for 10 minutes, the sections were then washed in phosphate buffered saline (PBS) for 5 minutes and dried around the tissue sections.

Antigen retrieval: Antigen retrieval was performed by immersing the slides in 10 mmol/l citrate buffer solution (pH 6.0) for 10 minutes at 100°C in microwave oven. The sections were then washed with PBS.

Blocking nonspecific staining: Incubation of sections with Ultra V block was done for 10 min to prevent non-specific background staining then followed by rinsing the sections with PBS.

Exposure to primary antibody: An overnight incubation of the sections with an antibody against Her2/neu was done at room temperature in a humid chamber (clone e2-4001+3B5, Neo-markers, Lab Vision Crop.).

Exposure to biotinylated secondary antibody: The sections were then washed with PBS and incubated with biotinylated goat anti-polyvalent (secondary antibody) for 10 min at room temperature followed by washing with PBS.

Exposure to streptavidin-biotin complex: The sections were then incubated with streptavidin peroxidase solution for 10 min at room temperature followed by washing with PBS.

Preparation of the working color reagent: The reaction products were visualized using 3-3-diamino-benzidine-tetrahydrochloride (DAB).

Color development: The sections were then counterstained with Mayer's hematoxylin,

dehydrated in alcohol and mounted in Dibutylphthalate Polystyrene Xylene (DPX).

Control of HER2/neu immunostaining: Positive and negative control slides were included within each session. Sections from the positive breast cancer (score 3) were used as positive controls, while negative controls were prepared by the omission of the primary antibody.

Evaluation of immunostaining: HER 2 expression was scored following the guidelines used (Bang *et al.,* 2010) as follows: no staining or no membranous staining of tumor cells was scored as "0", tumor cells with faint membrane staining irrespective of the percentage of tumor cells were scored as "1+", tumor cells with weak to moderate membrane staining irrespective of the percentage of tumor cells were scored as "2+", tumor cells with strong complete, basolateral, or lateral membrane reactivity irrespective of the percentage of tumor cells were scored as "3+". The study was approved by the research ethical committee of Faculty of Medicine, Tanta University following the ethical standards laid down in the 1964 Declaration of Helsinki

Statistical Analysis

Data were collected, tabulated, and analyzed using IBM SPSS software package version 20.0**.** According to the type of data, qualitative data was represented as number and percentage, quantitative continues group represented by mean ± SD. The following tests were used to test differences for significance; difference and association of qualitative variables by Chi-square test (X2). Differences between quantitative independent groups by T-test. The Mann-Whitney U and Kruskal Wallis tests were used to compare non-normal distribution data between two or multiple groups, respectively. ANOVA test was used for comparison among different times in quantitative data. The significance of the obtained results was judged at the 5% level.

RESULTS

Patients with esophageal adenocarcinoma were 5 males (71.4%) and 2 females (28.6%) with mean ages of 62.0 ± 6.58 years. Although patients were older than those with Barrett's esophagus and the control, yet, this difference was statistically non-significant (Table 1). The diagnosis of esophageal adenocarcinoma was based on endoscopic findings (Figure 2) and histopathological examination. It was found that one patient was classified as grade I, three patients grade II and three patients grade III (Figure 3).

In the control group: All of them were endoscopically normal (Figure 4) except 1 subject (10.0%) had non-specific gastritis. In Barrett's esophagus patients: 3 patients showed duodenal ulcer (13.0%), 7 showed hiatus hernia (30.4%), 1 patient had non-specific gastritis (4.3%). In esophageal adenocarcinoma patients: 1 patient showed duodenal ulcer (14.3%), 2 patients showed sliding hiatus hernia (28.6%), stricture was present in 2 patients (28.6%) and 1 patient presented with ulcerated mass (14.3%). There were significant differences between the control group and patients with GERD regarding endoscopic findings (P=0.0001) (Table 2).

Membranous expression of HER2 was scored from 0-3 according to the number of the positively stained cells and the intensity of staining. None of the 10 control subjects showed positive staining for HER2. In patients with Barrett's esophagus, weak positive staining (+1) of HER2 was detected in 39.1% (9/23) patients, moderate positive staining (+2) was detected in 30.4% (7/23) patients, strong positive staining (+3) was detected in 4.3% (1/23) patient, whereas 26.1% (6/23) were negative. In the esophageal adenocarcinoma group, the staining of HER2 in non-tumorous tissue was weak in 42.9% (3/7), whereas 57.1% (4/7) were negative. On the other hand, staining of HER2 was strong in 14.3% (1/7), moderate in 28.6% (2/7) and weak in 57.1% (4/7) of tumorous tissues. Thus, all cases of esophageal adenocarcinoma were positive for HER2 expression in tumorous tissue (100%).

There was a significant difference between the control group and patients with GERD regarding HER2 expression (P=0.001). HER2 expression was significantly increased in patients with Barrett's esophagus and those with esophageal adenocarcinoma (tumorous tissue) as compared to the control group (P=0.002 and

P=0.001, respectively). Moreover, HER2 expression was significantly higher in tumorous tissue than in Barrett's esophagus and non-tumorous tissue (P=0.038 and P=0.047, respectively) (Table 3 & Figure 5).

In patients with Barrett's esophagus, positive staining for HER2 was detected in 17 (73.9%) patients, whereas the remaining 6 (26.1%) patients were negative. As regards HER2 expression profile in patients with long-segment (10 patients), weak positive staining for HER2 was detected in 4 (40%) patients, moderate positive staining was observed in 3 (30%) patients, whereas the remaining 3(30%) were negative. As regards HER2 expression in those with short-segment (13 patients), weak positive staining for HER2 was present in 5 (38.5%) patients, moderate positive staining was detected in 4 (30.8%) patients, strong staining in 1 (7.7%) patient, whereas the remaining 3(23.1%) were negative but the differences were statistically non- significant (P=0.831). As regards HER2 expression in patients with intestinal metaplasia (16 patients), weak positive staining for HER2 was detected in 6 (37.5%) patients, moderate positive staining was observed in 4 (25%) patients, strong staining in 1 (6.3%) patient, whereas the remaining 5 (31.3%) were negative. As regards HER2 expression in those with cardiac/fundic metaplasia (7 patients), weak positive staining for HER2 was present in 3 (42.9%) patients, moderate positive staining was detected in 3(42.9%) patients, whereas the remaining 1 (14.3%) patient was negative, however, the differences were statistically non-significant. Regarding the degree of dysplasia in Barrett's esophagus patients, no HER2 immunostaining was detected in any of the 5 cases of negative dysplasia (Figure 6a). Of the 7 cases with low-grade dysplasia (Figure 6b), 3 (42.9%) were weakly positive for HER2, however, the remaining 4 (57.1%) cases were moderately positive. On the other hand, all cases of high-grade dysplasia (Figure 6c) were positive for HER2. There was a significant difference between Barrett's esophagus patients with positive HER2 expression and those with negative expression regarding the type of dysplasia (P=0.001) (Table 4 & Figure 7). As regards histopathological grading in esophageal adenocarcinoma patients,1 patient with grade I showed weak positive staining for HER2 (100%).

Table 1. Demographic data for all subjects

Sex	**Control (n=10)**		**GERD (n=30)**				**Total**		χ^2 **P**
			Barrett's esophagus (n=23)		**Esophageal adenocarcinoma (n=7)**				
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
Male	4	40.0	18	78.3	5	71.4	27	67.5	4.711
Female	6	60.0	5	21.7	2	28.6	13	32.5	0.095
	Control (n=10)		**Patients with GERD (n=30)**				**F**		**P**
			Barrett's esophagus (n=23)		**Esophageal adenocarcinoma (n=7)**				
Age (Years)									
Range	43-64		40-65		56-74		2.221		0.123
Mean±SD	54.20±6.53		55.87±8.62		62.00±6.58				
BMI (kg/m^2)									
Range	24.70-33.00		23.00-34.30		23.00-25.00		6.506		0.004*
Mean±SD	28.03±2.95		28.19±2.78		24.24±0.65				

Sex distribution, age and body mass index in control and patient groups. Non-significant (P>0.05) X^2, P: X^2 and P values for chi-square test.in control and patient groups.*Statistically significant (P<0.05) F, P: F and P values for ANOVA test

Table 2. Distribution of endoscopic findings among the studied groups

Endoscopic findings	Control (n=10)		GERD (n=30)				χ^2 P
			Barrett's esophagus (n=23)		Esophageal adenocarcinoma (n=7)		
	N	%	N	%	N	%	
Duodenal ulcer	0	0	3	13.0	1	14.3	47.615 0.0001*
Para-esophageal hiatus hernia	0	0	7	30.4	0	0	
Non-specific esophageal inflammation	0	0	0	0	1	14.3	
Non-specific gastric inflammation	1	10.0	1	4.3	0	0	
Sliding hiatus hernia	0	0	0	0	2	28.6	
Stricture	0	0	0	0	2	28.6	
Ulcerated mass	0	0	0	0	1	14.3	
No lesion	9	90.0	12	52.2	0	0	

*Statistically significant (P<0.05) X^2, P: X^2 and P values for chi-square test.

Table 3. HER2 expression profile among the studied groups

HER2 expression	Control (n=10)		GERD (n=30)					
			Barrett's esophagus (n=23)		Esophageal adenocarcinoma (n=7)			
					Tumorous tissue		Non-tumorous tissue	
	N	%	N	%	N	%	N	%
0	10	100	6	26.1	0	0	4	57.1
+1	0	0	9	39.1	4	57.1	3	42.9
+2	0	0	7	30.4	2	28.6	0	0
+3	0	0	1	4.3	1	14.3	0	0
χ^2 P	22.517 0.001*							

^{FE}P1	^{FE}P2	^{FE}P3	^{FE}P4	^{FE}P5	^{FE}P6
0.002*	0.001*	0.063	0.038*	0.261	0.047*

*Statistically significant (P<0.05) X^2, P: X^2 and P values for chi-square test, FE=Fisher's exact, P1: Control Vs Barrett's esophagus, P2: Control Vs Tumorous tissue .P3: Control Vs Non- tumorous tissue, P4: Barrett's esophagus Vs Tumorous tissue, P5: Barrett's esophagus Vs Non- tumorous tissue, P6: Tumorous Vs Non- tumorous tissue

Table 4. Relation between HER 2 expression values and mucosal affection with different degrees of dysplasia in patients with Barrett's esophagus

Variables	N	HER2 expression in patients with Barrett's esophagus (n=23)								χ^2	P
		Negative (n=6)		Positive (n=17)							
				+1(n=9)		+2(n=7)		+3(n=1)			
		N	%	N	%	N	%	N	%		
Type of segment											
Short	13	3	23.1	5	38.5	4	30.8	1	7.7	0.878	0.831
Long	10	3	30.0	4	40.0	3	30.0	0	0		
Type of metaplasia											
Cardiac/fundic	7	1	14.3	3	42.9	3	42.9	0	0	1.521	0.678
Intestinal	16	5	31.3	6	37.5	4	25.0	1	6.3		
Degree of dysplasia											
High grade dysplasia	5	0	0	1	20.0	3	60.0	1	20.0	29.275	0.001*
Indefinite	6	1	16.7	5	83.3	0	0	0	0		
Low grade dysplasia	7	0	0	3	42.9	4	57.1	0	0		
Negative	5	5	100	0	0	0	0	0	0		

*Statistically significant (P<0.05) X^2, P: X^2 and P values for chi-square test

Among cases with grade II adenocarcinoma, 2 patients were weakly positive (66.7%), while the remaining case was moderate (33.3%). On the other hand, cases of grade III showed weak positive staining in 1 patient, moderate in another one and strong in the remaining case (33.3% for each), however, the differences were statistically non-significant (Table 5).

HER2 expression in tumorous tissue was significantly affected by TNM stage in esophageal adenocarcinoma patients (P=0.030) However, no significant differences were detected in HER2 expression in tumorous tissue when the grade or the size of the tumors were considered.

DISCUSSION

The HER2 is a 1255 amino acid, 185 KDa transmembrane glycoprotein located at the long arm of human chromosome 17 (17q21) (Rubin & Yarden, 2001). HER2 is expressed in many tissues and regulates proliferation, adhesion, differentiation, and migration via activation of the RAS-MAPK and PI3K-AKT pathways (Steven & Danial, 2017). When HER2 is normally expressed, ligands that bind to the HER receptors form only a few HER2 heterodimers, and the responses to growth factors are relatively weak, resulting in the normal growth of cells. However, when HER2 is overexpressed as in cancer cells, multiple HER2 heterodimers are formed and cell signaling is stronger, resulting in enhanced responsiveness to growth factors and malignant transformation (Steven & Danial, 2017).

HER2 plays a role in the development and progression of several types of cancer, including breast, colorectal, and esophageal squamous cell carcinoma (Sergina & Moasser, 2007). Moreover, HER2 overexpression and amplification were detected in advanced gastric cancers (Gravalos & Jimeno, 2008), and effective targeting of HER2 was demonstrated using trastuzumab (Herceptin); a humanized monoclonal anti-HER2 antibody against the HER2 ectodomain and associated with clinical benefit compared with chemotherapy alone (Bang *et al.,* 2010). The status of HER2 expression/amplification and its association with clinicopathologic features and clinical outcome in esophageal adenocarcinoma remains unclear. Also, the correlation of HER2 expression with early neoplastic development in Barrett's esophagus has not been well explored (Tanaka *et al.,* 2012, Yoon *et al.,* 2012 b and Nagaraja *et al.,* 2016).

This study was conducted to evaluate the role of HER 2 expression as a potential marker for the progression of Barrett's esophagus to esophageal adenocarcinoma and to examine its relationship with the clinicopathological features of the patients. In the present study, endoscopic findings in Barrett's esophagus patients revealed significant differences between the control group and patients with GERD regarding endoscopic findings (P=0.0001).

Table 5. HER2 expression in correlation with histopathological findings (grades) in esophageal adenocarcinoma patients

Histopathological findings (Grades)	HER2 expression in esophageal adenocarcinoma patients (n=7)									
	0		+1 (n=4)		+2 (n=2)		+3 (n=1)		Total	
	N	%	N	%	N	%	N	%	N	%
Grade I	0	0	1	100	0	0	0	0	1	14.3
Grade II	0	0	2	66.7	1	33.3	0	0	3	42.9
Grade III	0	0	1	33.3	1	33.3	1	33.3	3	42.9
χ^2	2.333									
P	0.675									

The results were statistically significant ($p<0.05$) x^2, p: x^2 and p values for chi-square test

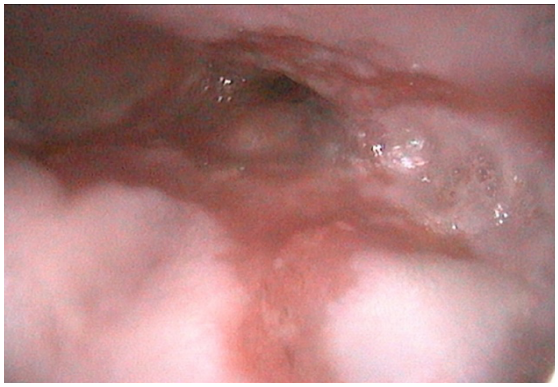


Figure 1. Barrett's esophagus with tongue-like projections of reddish-brown mucosa at the distal end of the esophagus

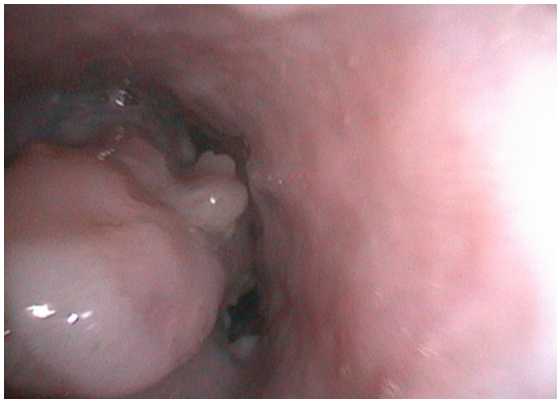


Figure 2. Barrett's esophagus progressed to adenocarcinoma.

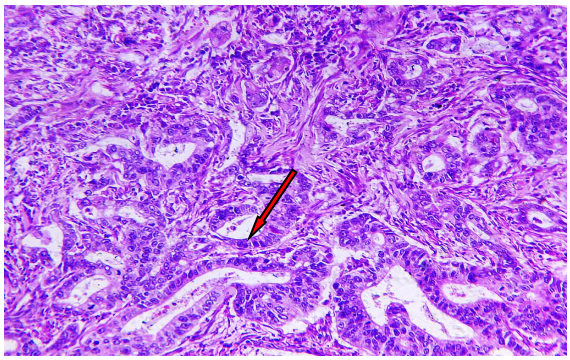


Figure 3. Esophageal adenocarcinoma grade III arising in the metaplastic glands of Barrett's esophagus (red arrow) [H & E. X 200].

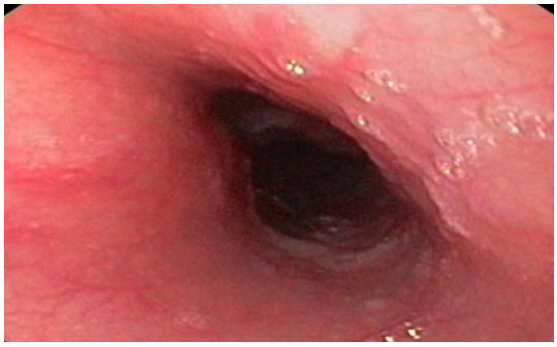


Figure 4. Normal esophagogastroduodenoscopy

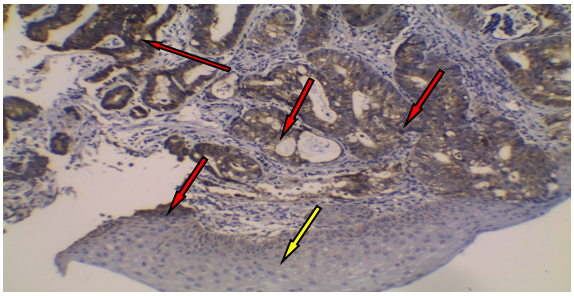
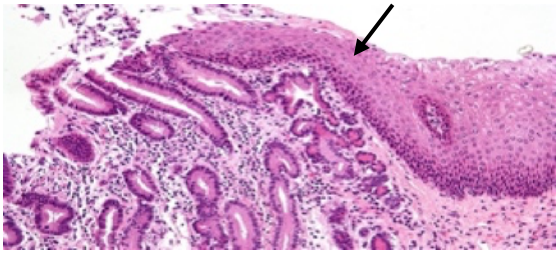
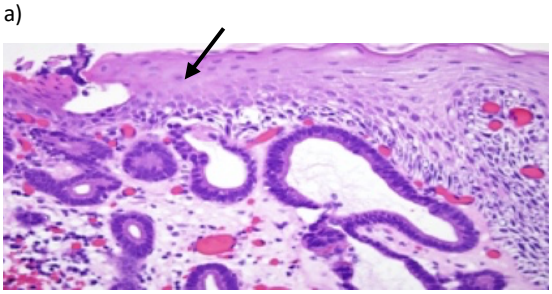


Figure 5. Esophageal adenocarcinoma grade II showing score +3 HER2 expression in tumorous tissue (red arrows) while the staining of HER2 in non-tumorous tissue were negative (yellow arrow). [ABC. X200]



a)



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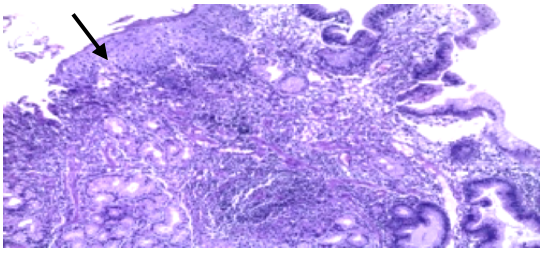

c)

Figure 6. Barrett's turned into different degrees of metaplasia: a) barrett's esophgous with negative dysplasia, b) low grade dysplasia, c) high grade dysplasia.

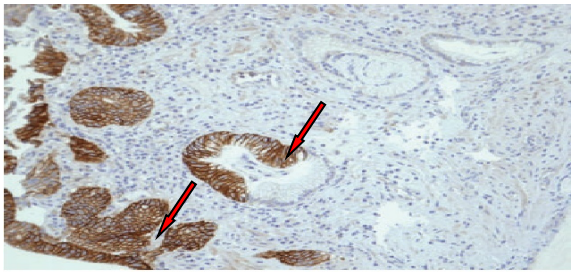

Figure 7. Barrett's esophagus with high grade dysplasia showing score +3 HER2 expression in the dysplastic glands (red arrows) while the glands with negative dysplasia show score 0 [ABC.X200].

There were no significant differences between Barrett's esophagus patients or esophageal adenocarcinoma patients with positive and those with negative fecal occult blood tests regarding endoscopic findings. The present study indicates that even GERD patients with negative fecal occult blood test should undergo follow-up by upper endoscopy for early detection of BE and EAC. The results of endoscopic examination of our patients with BE revealed the presence of long-segment BE in 43.5% and short-segment BE in the remaining 56.5%. Our results demonstrated that patients with long-segment BE were older and had a longer duration of GERD symptoms than those with short-segment but the differences did not reach a significant level.

Regarding histopathological examination of mucosal biopsies in the current study, the findings showed that 69.6% of Barrett's esophagus patients had intestinal metaplasia, while the remaining 30.4% had cardiac/fundic type of metaplasia. On the other hand, 21.7% of Barrett's esophagus patients had high-grade dysplasia, 26.1% showed indefinite dysplasia, 30.4% had low-grade dysplasia, while the remaining 21.7% patients were negative for dysplasia. In addition, 14.3% of esophageal adenocarcinoma patients were classified as grade I, 42.9% had grade II, while the remaining 42.9% patients were classified as grade III.

In the present study, none of the control subjects showed positive staining for HER2. In BE patients, 39.1% showed weak positive staining (+1), moderate staining (+2) was detected in 30.4%, strong staining (+3) in 4.3%, whereas 26.1% were negative. On the other hand, the staining of HER2 in non-tumorous tissue of esophageal adenocarcinoma was weak in 42.9%, while 57.1% were negative. Regarding the tumorous tissue, staining of HER2 was strong in 14.3%, moderate in 28.6% and weak in 57.1%. Thus, all cases of esophageal adenocarcinoma were positive for HER2 expression in tumorous tissue.

HER2 expression was significantly increased in patients with Barrett's esophagus and those with esophageal adenocarcinoma (tumorous tissue) as compared to the control group (P=0.002 and 0.001, respectively). Moreover, HER2 expression was significantly higher in tumorous tissue than in Barrett's esophagus and in non-tumorous tissue (P=0.038 and 0.047, respectively). Regarding the degree of dysplasia, there was a significant difference between Barrett's esophagus patients with positive HER2 expression and those with negative expression (P=0.001).

As regards histopathological grading in esophageal adenocarcinoma patients, grade I showed weak positive staining for HER2. Among cases with grade II, 66.7% were weakly positive, while the remaining 33.3% was moderate. On the other hand, grade III showed weak, moderate, and strong positive staining in 33.3% for each, but the differences were statistically non-significant. HER2 expression in tumorous tissue was significantly affected by TNM stage (P=0.030), however, no statistically significant differences were detected when the tumor grade or size was considered. These findings are supported by Gravalos and Jimeno (2008), who observed that HER2 overexpression was most commonly found in gastro-esophageal junction tumors and tumors having intestinal-type histology. HER2 overexpression is reported in esophageal cancers, with a tendency towards higher rates of positivity in adenocarcinoma compared to squamous cell carcinomas (Flejou, Paraf & Muzeau, 1994).

Yoon et al. (2012b), found HER2 positivity in 17% of esophageal adenocarcinoma patients and it was significantly associated with lower tumor grade, less invasiveness, fewer malignant nodes, and the presence of adjacent Barrett's esophagus. In EAC with Barrett's esophagus, HER2 positivity was significantly associated with improved overall survival independent of pathologic features but was not prognostic among EAC without BE. However, another study found that HER2 heterogeneity among HER2 amplified EAC was an independent predictor of worse cancer-specific survival (Yoon, Shi & Sukov, 2012a). Apart from EAC, HER2 overexpression was also found to be a negative predictor of survival in esophageal squamous cell carcinoma (Zhan *et al.,* 2012).

Similarly, Rossi *et al.* (2009), showed that HER2 amplification/overexpression was correlated to dysplasia in BE and with progression to more advanced step (EAC) and that time to progression was shorter in HER2 positive than in HER2 negative groups. Thus, they cannot rule out a definitive causative role for HER2 in the pathogenesis of esophageal adenocarcinoma for which further studies and larger series of patients are required. These findings are in agreement with Langer, Von-Rahden & Nahrig, 2006, who reported that 21% of cancer patients showed strong staining (16% 2+, 5% 3+) and that strong staining was evident in 20–25 % of esophageal adenocarcinoma. Similar findings were also presented by Wang, Wu & Choi (2007). Cronin *et al.,* 2011 found that HER2 expression was shown to increase 13-fold in esophageal adenocarcinoma tissue compared to the BE tissues, suggesting that 35 % of high-grade dysplasia and 80 % of adenocarcinoma tissues showed HER2 overexpression. Furthermore, as HER2 is a membranous protein expressed on the luminal surface of the esophageal mucosa, it may also be a useful target for biopsy guidance during endoscopy to identify dysplastic lesions.

Meanwhile, Tanaka *et al.,* 2012 observed that 27% of Barrett's adenocarcinomas in Japanese patients were HER2 positive. However, there were no prognostic differences between the HER2 positive and HER2 negative cases. The frequency of protruding lesions was significantly higher in the HER2 positive than in HER2 negative cases. Thus, protruded-type lesions can indicate HER2 positive status. However, it is important to note that another report has not linked HER2 expression to neoplastic development in BE (Van Dekken, Hop & Tilanus, 2008). The discrepancy between these findings may arise from several sources, e.g., tumor grade/stage, differential patient treatment, antibody efficiency, scoring method, and other methodological aspects related to immunohistochemistry.

On the other hand, Rygiel *et al.,* 2008 demonstrated that metaplastic Barrett's epithelium has been shown to lack HER2 expression and/or amplification, and the frequency of HER2 expression or amplification in BE- associated dysplasia is not well understood but may increase. Although the explanation for the association of HER2 with better clinical outcome remains unknown, a subtype of HER2 positive breast cancers is associated with an increased inflammatory or immune cell infiltration that showed a substantially improved prognosis as compared with other HER2 positive breast cancers, with potentially improved response to neoadjuvant trastuzumab-based chemotherapy (Pupa *et al.,* 1996). In this context, it is noteworthy that a link in the development and/or progression of BE with chronic inflammation and the immune response has been reported (Moons *et al.,* 2005).

Recently, Nagaraja *et al.,* 2016 detected a 9.9% HER2 positivity rate among patients with esophageal carcinoma but there was no significant overall relationship between HER2 status and survival. The HER2-positive tumors were more likely to occur in men, smokers, non-alcoholics, non-diabetics and patients with Barrett's esophagus. These tumors were more likely to be in the lower esophagus, well to moderately differentiated adenocarcinomas and to be early stage. With respect to HER2 status in gastro-esophageal carcinogenesis, Fassan *et al.* (2012), reported that HER2 overexpression was seen in low-grade and high-grade dysplasia (both gastric and esophageal), and increased significantly from low-grade to high-grade dysplasia, and adenocarcinoma. The results obtained provide evidence for the early involvement of HER2 dysregulation in the neoplastic transformation of both gastric and esophageal metaplastic mucosa, and these data are supported by some other studies (Villanacci, Rossi & Grisanti, 2008, Rossi *et al.,* 2009 and Rossi, Villanacci & Bassotti, 2010).

As HER2 is frequently overexpressed in human tumors, HER2 is being investigated as a target for cancer therapy. Its localization at the cell surface makes it an easy target to access. A wide range of therapeutic strategies targeting breast tumors that overexpress HER2 have been investigated, including tyrosine kinase inhibitors, antisense approaches, designed to down-regulate expression of the HER2 gene, and immunization to actively boost anti-HER2

responses. In addition, selective targeting can be achieved using monoclonal antibodies directed against the extracellular domain of the HER2 protein. As HER2 is expressed only in low levels in normal tissue, this permits a suitable therapeutic window to minimize damage to normal cells (Sergina & Moasser, 2007).

In addition, targeted anti-HER2 therapy is now available and showing some initial promise in treating esophageal adenocarcinoma (Uzunoglu, Koenig & Icbicki, 2014), coupling HER2 diagnostics to this novel treatment modality may slow neoplastic progression in some patients. Some limitations in our study should be addressed. The total number of patients was small. 24 hours PH monitoring was not performed. Special techniques like chromoendoscopy, narrow band endoscopy and endoscopic ultrasound are better options but they are not available at the time of the study.

CONCLUSION

Once the diagnosis of GERD has been established, it is necessary to examine the patient by upper GI endoscopy and mucosal biopsies to early detect Barrett's esophagus and esophageal adenocarcinoma. High HER2 expression levels correlated well with the degree of dysplasia in Barrett's esophagus patients and its progression to esophageal adenocarcinoma. HER2 expression may be regarded as a potential biomarker for early detection of esophageal adenocarcinoma. HER2 expression may have an important role in esophageal carcinogenesis and may be regarded as a useful target for elucidating the molecular mechanisms associated with cancer. Clearly, further well-designed prospective studies are required to prove this hypothesis.

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