Original Research Article

The Expression of E-cadherin & HER2 in Gastric Carcinoma

Abstract:

Background and Aim: Cell adhesion molecules (often referred to as cadherins) are glycoproteins found in the cell membrane. They regulate biological processes such as cell migration, differentiation, proliferation, and death (apoptosis). Her-2 is a proto-oncogene that belongs to the EGFR protein family. In cellular processes such as cell growth, regulates cellular activities such as proliferation, differentiation, and survival. This study aimed to determine the expression of E-cadherin and HER2 in the available histologic subtypes and evaluate if there is a correlation between E-cadherin and HER2 immunohistochemical expression in gastric carcinoma.

Subjects & Methods: A total of 50 cases of stomach cancer were included in this study, all of which were obtained retrospectively between January 2017 and January 2020 from the Pathology Department, Tanta University, and Tanta Cancer Center archives. The samples were obtained from gastroplastectomy specimens then stained using the immunostaining approach described as follows: Deparaffinization and rehydration followed by using 3-hydroxy-4-napthylbenzaldehyde (3-OH-4-NHB) as a starting material then a smorgasbord of antigens then exposing to primary antibodies then exposing to a secondary biotinylated antibody, after that identifying of enzymes using streptavidin-labeled enzymes then preparing a color working reagent and last complexity in the development of color.

Results: E-cadherin was statistically significantly high in tumors exhibiting aggressive clinicopathologic characteristics specifically in males, all histopathologic variants (except for poorly differentiated tubular adenocarcinoma and signet ring carcinoma), N stages, M stages, higher in absent vascular invasion cases but lower in present cases and the same with perineural invasion cases. Tumor site had no significant impact on the levels of E-cadherin. HER2 showed statistically insignificant correlations with all factors. E-cadherin and HER2 expression showed statistically insignificant relationship.

Conclusion: Elevated E-cadherin expression is associated with tumors that exhibit aggressive clinicopathologic characteristics. HER2 was expressed positively in low-grade variants, but

Comment [U1]: To long summarize important finding

no correlation was established with clinical characteristics. E-cadherin and HER2 expression have no discernible relationship.

Keywords: E-cadherin expression, HER2 expression, gastric carcinoma

Introduction

Gastric cancer is the fifth most commonly diagnosed cancer in the world, and the seventh most prevalent. In Egypt, gastric cancer is the 9 the most common cancer representing 1.7% of total malignancy in males [1, 2].

Numerous precancerous conditions, such as atrophic gastritis, intestinal metaplasia, gastric ulcers, gastric polyps, and previous stomach surgery, have all been implicated in the development of gastric cancer [3].

The incidence of gastric cancer appears to be reducing as a result of improved eating habits, food storage, less smoking, increased sanitation, and a decreased rate of H. pylori infection transmission [4].

Gastric cancer treatment requires a multidisciplinary strategy that includes surgery, adjuvant chemotherapy, and neoadjuvant chemotherapy. The most common treatment is surgery, which has a high success rate. Adjuvant chemotherapy improves overall survival more than surgery alone. A recent study demonstrated the efficacy of neoadjuvant chemotherapy, resulting in an improved overall prognosis [5].

Cell adhesion molecules (often referred to as cadherins) are glycoproteins found in the cell membrane. They regulate biological processes such as cell migration, differentiation, proliferation, and death (apoptosis). E-cadherin (epithelial type) and N-cadherin (extracellular type) are the most extensively studied cadherins (neural type). The epithelial cell adhesion molecule (E-CAM) is a calcium-dependent cell adhesion molecule that has been found to be overexpressed in a variety of cancers, including those of the large bowel, lung, and prostate. Numerous studies have established a link between the degree of E-cadherin expression and tumor cells' chemotherapeutic sensitivity [6].

Her-2 is a proto-oncogene that belongs to the EGFR protein family. In cellular processes such as cell growth, regulates cellular activities such as proliferation,

differentiation, and survival. Increased expression could induce uncontrolled cell proliferation and tumor development [7].

HER2 has been detected in a wide variety of human tissues, including the breast, gastrointestinal system, kidney, and heart. Trastuzumab is an anti-Her2-antibody that is used as a predictive biomarker for Trastuzumab therapy. Her2 positive cancers are treated with regular chemotherapy in conjunction with this biomarker. Due to the high cost and potential side effects, it is critical to select the appropriate patients for Trastuzumab therapy [8].

This study aimed to determine the expression of E-cadherin and HER2 in the available histologic subtypes and evaluate if there is a correlation between E-cadherin and HER2 immunohistochemical expression in gastric carcinoma.

Subjects and Methods

A total of 50 cases of stomach cancer were included in this study, all of which were obtained retrospectively between January 2017 and January 2020 from the Pathology Department, Tanta University, and Tanta Cancer Center archives. The samples were obtained from gastroplastectomy specimens.

The research was authorized by the Tanta University Ethics Committee and all subjects received signed written informed consent.

The following activities were completed in each case:

- Patient demographic data, tumor site, lymph node status and metastasis were documented.
- Histopathologic specimens were produced.

The cells were stained using the immunostaining approach described below:

a) Deparaffinization and rehydration

b) Using 3-hydroxy-4-napthylbenzaldehyde (3-OH-4-NHB) as a starting material

c) A smorgasbord of antigens

d) Exposure to primary antibodies

e) The opportunity to be exposed to a secondary biotinylated antibody

f) Identification of enzymes using streptavidin-labeled enzymes

g) Preparation of a color working reagent

h) Complexity in the development of color

Interpretation of E-cadherin immunostaining

The level of E-cadherin expression is determined by the intensity of brownish membranous staining and the percentage of positive cells. The following conclusions were drawn from this report on tumor cell reactivity cutoff values: 1 (low), 2 (moderate), and 3 (high), with a positive reaction limit of 5% (relative to tumor cells).

One (6–25% of cells were positive), two (26–50% of cells were positive), three (51–75% of cells were positive), and four (>75% of cells were positive). The final staining score was calculated by multiplying the intensity and percentage values, with lower intensity and percentage values indicating low staining and greater intensity and percentage values indicating high staining.

Interpretation of HER2 immunostaining

A brownish membranous staining of malignant cells was discovered in the area where HER2 expression was detected. I submitted the following scores: 0; 0% of tumor cells stained with the membrane. The term "feeble/brief membrane reactivity" refers to the fact that practically all cancer cells have a 10% faint membrane-membrane reactivity. Complete staining of the basolateral or lateral membranes of at least 10% of tumor cells in the moderate to strong range. Inclusion of more than 10% of cancer cells results in a significant increase in basolateral or lateral membranous reactivity in at least 3% of cancer cells. For IHC 0 and 1, Her2 protein expression was assigned a score of zero, whereas values of 0 and 3 were judged ambiguous and positive for HER2 expression, respectively [9].

Statistical Analysis

Statistical analysis was done using SPSS (Statistical Package for Social Science) version 21. For quantitative data, the range, mean, and standard deviation were established. Quantitative data were quantified using numbers and percentages. When the Chi-square test proved inapplicable, the Monte Carlo and Fisher Exact tests were used. Mann-Whitney When two groups were compared, the U-test was used; when more than two groups were compared, the Kruskal Wallis test was used. Spearman's formula was used to do the correlation study. At the significance level of p0.05, significance was determined.

Results

There were 30 (60%) males and 20 females (40%) ranging in age from 32 to 73 years. While eighty percent of patients were over fifty, only ten percent were under fifty. Of the fifty stomach tumors analyzed, 48% were detected in the pylorus, 34% in the cardia, 10% in the fundus, and 8% in the stomach body. **Table 1**

These analyzed examples were classified as follows: 22 incidences (44% tubular adenocarcinoma, 3 instances (6%) papillary adenocarcinoma, 14 instances (28% poorly cohesive carcinoma, 7 instances (14% mucinous carcinoma, 3 instances (6%) mixed carcinoma, and one incident (2%) carcinoma with lymphoid stroma. **Table 1**

Adenocarcinomas were well-differentiated in 12% of cases. **Table 1**

Twenty-two patients with tubular adenocarcinoma were investigated, 4 of whom had low-grade malignancy, 10 of whom had moderate-grade malignancy and 8 of whom had low-grade malignancy (high grade). 3 cases of papillary adenocarcinoma were evaluated, with one being well-differentiated (low-grade), and the other 2 being moderately differentiated (low grade). 3 cases of adenomatous adenocarcinoma were investigated. One example had a high degree of differentiation (low-grade), whereas the other 2 had a moderate degree of differentiation (low grade). There was no grading conducted on the remaining cases. **Table 1**

a- T stage (depth of invasion):

In T2, the tumor penetrated the muscularis propria in 16 individuals (about 8%). Over three-quarters (76%) of T3 cases involved tumors that had invaded the subserosal connective tissue but did not infiltrate other adjacent structures. 5 (10%) of the cases were T4 tumors with infiltrations in the serosa or adjacent tissues. **Table 1**

b- Lymph node metastasis (N stage):

Ten instances (20%) lacked indications of metastasis (N0). In fifteen cases (30% of 1-2 lymph nodes in the regional lymph system), metastases were detected (N1). Seventeen patients (34% of the total) demonstrated metastases in three to six lymph nodes (N2). There were eight occurrences (16 percent of the total) of metastasis in more than seven lymph nodes. **Table 1**

c- Distant metastasis (M stage):

Twelve patients (24 percent of the entire group) had metastases found; 38 patients (76 percent of the total group) had no distant metastases reported by clinic pathology reports. **Table 1**

Tumor-derived emboli were detected in around 36% of cases where tumor-derived emboli were detected in the lumen of blood vessels. **Table 1**

Perineural invasion was discovered in nineteen instances (38%). **Table 1**

E-cadherin is visualized on the cell membrane as a membranous brownish staining. Of the 50 stomach cancer patients analyzed, 62% (31 cases) had a high score (6-12) and 38% (19 cases) received a poor score (1-5). **Table 1**

HER2 expression is characterized by a membranous brownish staining. Four of fifty stomach cancer cases tested had a positive (3) score, whereas the remaining forty-six cases (92%) had a negative (0) score (0).

Table 1: Demographic data, tumor site, distribution of histopathologic variants and their grading, T stage, N stage, M stage, vascular and perineural expression and E-cadherin expression and HER2 expression of the studied cases

	N	%
Sex		
Male	30	60
Female	20	40
Age		
< 50	10	20
≥ 50	40	80
Tumor site		
Body	4	8
Cardia	17	34
Fundus	5	10
Pylorus	24	48
Distribution of histopathologic variants		
Tubular adenocarcinoma	22	44.0
Papillary	3	6.0
Signet ring	14	28.0
Mucinous	7	14.0

Mixed	3	6.0
Carcinoma with lymphoid stroma	1	2.0
Histopathologic grading		
Tubular adenocarcinoma well differentiated (low grade)	4	8
Tubular adenocarcinoma moderately differentiated (low grade)	10	20
Tubular adenocarcinoma poorly differentiated (high grade)	8	16
Papillary adenocarcinoma well differentiated (low grade)	1	2
Papillary adenocarcinoma moderately differentiated (low grade)	2	4
Mixed adenocarcinoma well differentiated (low grade)	1	2
Mixed adenocarcinoma moderately differentiated (low grade).	2	4
Other types (not graded)	22	44
T stage		
2	8	16
3	37	74
4	5	10
N- stage		
0	10	20
1	15	30
2	17	34
3	8	16
M stage		
0	38	76
1	12	24
Vascular invasion		
Present	18	36
Absent	32	64
Perineural invasion		
Present	19	38
Absent	31	62
E-cadherin expression		
Low	19	38

High	31	62
HER2 expression		
+ve	4	8
-ve	46	92
Total	50	100.0

T stage: tumor stage, N: Lymph node metastasis, M stage: Distant metastasis

Relationship between E-cadherin expression & different factors:

Twelve (60%) of the twenty female patients had abnormal E-cadherin expression, whereas seven (23%) of the thirty male patients had abnormal E-cadherin expression. Statistical analysis verified this association where it showed statistically significant increase in abnormal (high) E-cadherin expression than low. **Table 2**

Of the four cases (8%) discovered in the stomach body, two (4%) had high E-cadherin scores, while the remaining two (4%) had low E-cadherin scores. 22% of the cardia samples had high levels of E-cadherin, whereas 12% exhibited low levels of E-cadherin. Five instances were discovered in the gastric fundus. 4 of these cases (8%) had increased E-cadherin levels, while just one (2%) had decreased E-cadherin values. Within the pylorus 14 (28%) were exhibiting elevated E-cadherin levels and the other 10 (20%) exhibited decreased E-cadherin levels. This correlation was statistically negligible. **Table 2**

All four patients with tubular well-differentiated adenocarcinoma (100%) had high E-cadherin expression. In 70% of cases of moderately differentiated adenocarcinoma, E-cadherin expression was high, whereas in 30% of cases, E-cadherin expression was low. In 25% of cases of poorly differentiated tubular adenocarcinoma, E-cadherin expression was high, whereas it was low in the remaining cases. 22-28 Illustration. All three individuals with papillary cancer had high E-cadherin score. A high E-cadherin score was reported in five of the fourteen instances (35.8 percent of all poorly cohesive carcinoma with signet ring adenocarcinoma, specifically signet ring carcinoma). Meanwhile, a low E-cadherin score was reported in nine cases (64.2% of all poorly cohesive carcinoma with signet ring adenocarcinoma, i.e., signet ring carcinoma). E-cadherin is expressed in 85.7% of cases of mucinous adenocarcinoma but is absent in 14.2% of these tumors. a self-evident conclusion. All three patients diagnosed with mixed adenocarcinoma (100%) had high E-cadherin score.

It is remarkable in that only one case of cancer exhibits lymphoid stroma (100% lymphoid stroma). The correlation was statistically significant. **Table 2**

Six of the eight T2 instances had a positive E-cadherin score, whereas the remaining two had a negative E-cadherin score. There were 22 cases of high E-cadherin and 15 cases of low E-cadherin in 37 individuals with T3 stage. According to the data, 60% of 5 T4 patients had high E-cadherin scores, whereas 40% had low E-cadherin levels. This association was statistically insignificant. **Table 2**

E-cadherin levels were elevated in all N0 patients. 60% had a high level of E-cadherin expression, while 40% had a low level of E-cadherin expression. Eight of the seventeen N2 cases had a positive E-cadherin value, whereas the remaining nine had a negative E-cadherin value. N3 stage accounts for about half of all E-cadherin-negative breast cancer cases; of the remaining four cases, approximately half (50%) of patients had a high E-cadherin score, while the other half (50%) had a low E-cadherin score. Statistical analysis verified this association. **Table 2**

Regarding M stage, 56% of M0 patients exhibited elevated E-cadherin levels, while 20% had decreased E-cadherin levels. For the M1 stage, 6% of cases had a high E-cadherin score, whereas 9% had a low E-cadherin score. Statistical analysis verified this association. **Table 2**

Twelve percent of patients with vascular invasion had high E-cadherin scores, whereas twenty-four percent had low E-cadherin values. 25 patients (50%) showed elevated E-cadherin levels, while 7 cases (14%) had decreased E-cadherin levels. Statistical analysis verified this association. **Table 2**

Nineteen cases of perineural invasion (18%) had a high E-cadherin score, while ten cases (20%) had a low E-cadherin score. Out of 31 cases without perineural invasion, 22 (44%) had a high E-cadherin score, whereas the remaining 9 (18%) had a low E-cadherin score. This correlation was statistically negligible. **Table 2**

Table 2: Correlation between E-cadherin expression & different factors

	E-cadherin		Total	P value
	Low	High		
Sex				

Male	**N**	7	23	30	**0.009***
	%	14.0%	46.0%	60.0%	
Female	**N**	12	8	20	
	%	24.0%	16.0%	40.0%	
Tumor site					
Body	**N**	2	2	4	**0.772**
	%	4.0%	4.0%	8.0%	
Cardia	**N**	6	11	17	
	%	12.0%	22.0%	34.0%	
Fundus	**N**	1	4	5	
	%	2.0%	8.0%	10.0%	
Pylorus	**N**	10	14	24	
	%	20.0%	28.0%	48.0%	
Histopathologic variants					
Tubular well differentiated	**N**	0	4	4	**0.015***
	%	.0%	8.0%	8.0%	
Tubular moderately differentiated	**N**	3	7	10	
	%	6.0%	14.0%	20.0%	
Tubular poorly differentiated	**N**	6	2	8	
	%	12.0%	4.0%	16.0%	
papillary	**N**	0	3	3	
	%	0.0%	6.0%	6.0%	
Mucinous	**N**	1	6	7	
	%	2.0%	12.0%	14.0%	
Mixed	**N**	0	3	3	
	%	.0%	6.0%	6.0%	
Signet ring	**N**	9	5	14	
	%	18.0%	10.0%	28.0%	
Carcinoma with lymphoid stroma	**N**	0	1	1	
	%	.0%	2.0%	2.0%	
T stage					
2	**N**	2	6	8	
	%	4.0%	12.0%	16.0%	
3	**N**	15	22	37	
	%	30.0%	44.0%	74.0%	
4	**N**	2	3	5	
	%	4.0%	6.0%	10.0%	
N stage					
0	**N**	0	10	10	**0.041***
	%	.0%	20.0%	20.0%	
1	**N**	6	9	15	
	%	12.0%	18.0%	30.0%	
2	**N**	9	8	17	
	%	18.0%	16.0%	34.0%	
3	**N**	4	4	8	
	%	8.0%	8.0%	16.0%	
M stage					

0	**N**	10	28	38	**0.002***
	%	20.0%	56.0%	76.0%	
1	**N**	9	3	12	
	%	18.0%	6.0%	24.0%	
Vascular invasion					
Present	**N**	12	6	18	**0.002***
	%	24.0%	12.0%	36.0%	
Absent	**N**	7	25	32	
	%	14.0%	50.0%	64.0%	
Perineural invasion					
Present	**N**	10	9	19	**0.095**
	%	20.0%	18.0%	38.0%	
Absent	**N**	9	22	31	
	%	18.0%	44.0%	62.0%	

*: statistically significant P value, T stage: tumor stage, N: Lymph node metastasis, M stage: Distant metastasis

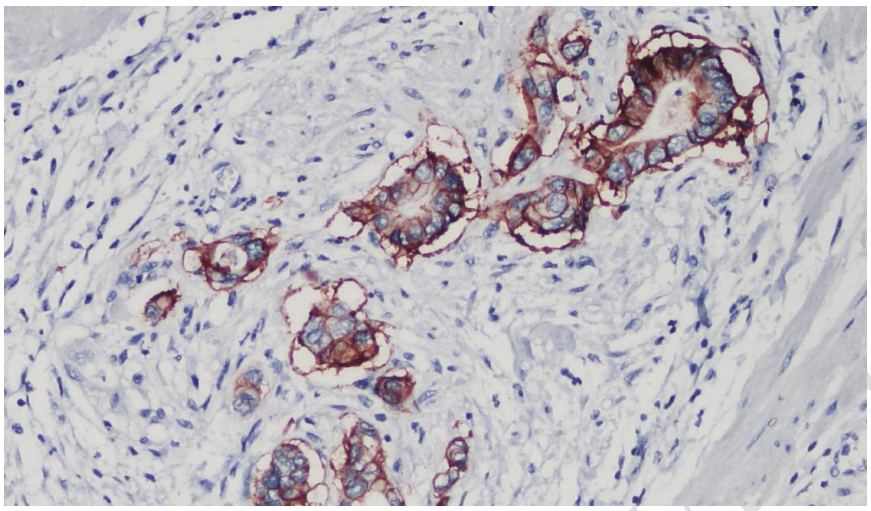

Figure 1: **Tubular adenocarcinoma, well differentiated, showing high membrance expression of E-cadherin score 12,(Streptavidin -biotin x200).**

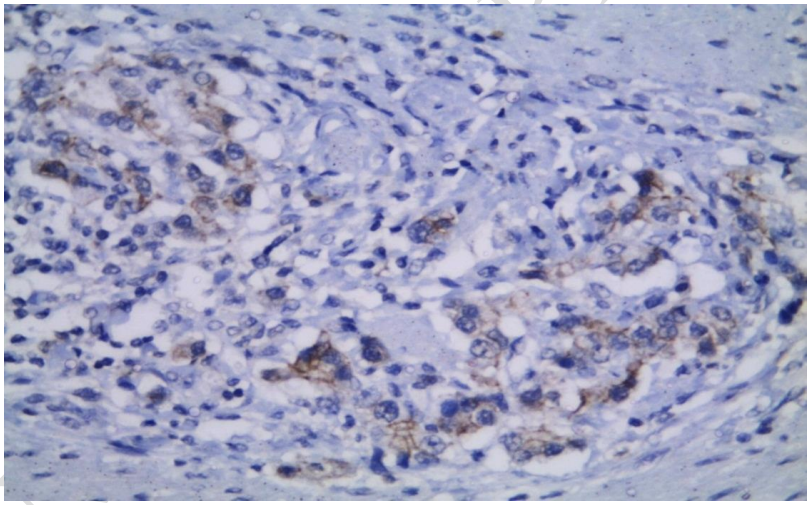

Figure 2: **Tubular adenocarcinoma; poorly differentiated showing low E-cadherin expression score 2, (Streptavidin-biotin x400)**

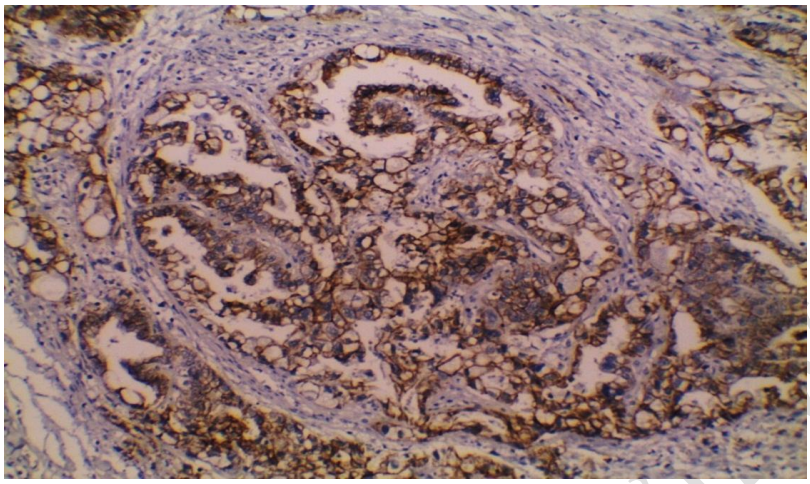

Figure 3: Papillary adenocarcinoma, well differentiated showing high E-cadherin expression score 12, (Streptavidin-biotin x200).

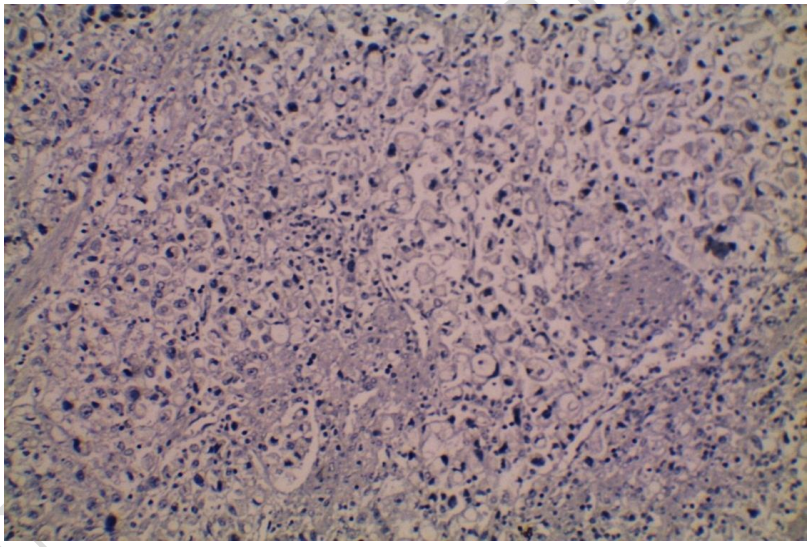

Figure 4: Signet ring carcinoma, showing low E-cadherin expression score 1, (Streptavidin-biotin x200)

Relationship between HER2 expression & different factors:

Men were affected at a rate of 6.6 percent, while women were affected at a rate of 2.0 percent (10%). It was statistically insignificant. A positive score of 3 was obtained in four of the forty patients (10%) who were over the age of 50. **Table 3**

The three instances discovered in the gastrointestinal tract did not test positive for HER2. HER2 was found in the cardia in 11.8 percent of instances (i.e., one case out of 17); in the fundus in 5.2 percent of cases (i.e., one case out of 24); and in the pylorus in 4.2 percent of cases (i.e., one case out of 24). That was without a statistically significant outcome. **Table 3**

HER2 positivity was identified in one of two patients with well-differentiated tubular adenocarcinoma (2 percent). Two of the three patients with moderately differentiated tubular adenocarcinoma had HER2 positivity (6 percent). All other possibilities were given a score of zero (0). It was statistically insignificant. **Table 3**

Positive HER2 test findings were detected in 25% of those with T2 stage cancer and in 5.4% of those with T3 stage cancer. **Table 3**

HER2 positivity was detected in one case (10%) of all patients with N0-stage disease and three instances (17.6 percent) of all cases with N2-stage disease in this investigation but did not reach statistical significance. **Table 3**

Her2 positivity was detected in four out of 38 (11.5%) patients with M0 stage illness, although this result lacked statistical significance. **Table 3**

HER2 was positive in 1 (1 out of 5.5 percent) and negative in 3 (3, 9.4%) of the 5.5 percent of cases with vascular invasion, with no statistical significance. **Table 3**

Three patients (10.5 percent) showed HER2-positivity with perineural invasion, while two cases (6.5 percent) had HER2-positivity without perineural invasion, both with non-statistically significant results. **Table 3**

Table 3: Correlation between HER2 expression & different factors

		HER2		Total	P value
		+ve	-ve		
Sex					
Male	**N**	2	28	30	**0.670**
	%	4.0%	56.0%	60.0%	
Female	**N**	2	18	20	
	%	4.0%	36.0%	40.0%	
Age					
< 50	**N**	0	10	10	**0.297**
	%	.0%	20.0%	20.0%	

≥ 50	**N**	4	36	40	
	%	8.0%	72.0%	80.0%	
Tumor site					
Body	**N**	0	4	4	**0.545**
	%	.0%	8.0%	8.0%	
Cardia	**N**	2	15	17	
	%	4.0%	30.0%	34.0%	
Fundus	**N**	1	4	5	
	%	2.0%	8.0%	10.0%	
Pylorus	**N**	1	23	24	
	%	2.0%	46.0%	48.0%	
		HER2		**Total**	**P-value**
		+ve Score 3	**-ve**		
Tubular adenocarcinoma well differentiated	**N**	1	3	4	**0.373**
	%	2 %	8.0%	8.0%	
Tubular adenocarcinoma moderately differentiated	**N**	3	7	10	
	%	6.0%	14.0%	20.0%	
Tubular adenocarcinoma poorly differentiated	**N**	0	8	8	
	%	.0%	16.0%	16.0%	
Papillary	**N**	0	3	3	
	%	.0%	6.0%	6.0%	
Mucinous	**N**	0	7	7	
	%	0.0%	14.0%	14.0%	
Mixed	**N**	0	3	3	
	%	.0%	6.0%	6.0%	
Papillary	**N**	0	14	14	
	%	0.0%	28.0%	28.0%	
Carcinoma with lymphoid stroma	**N**	0	1	1	
	%	.0%	2.0%	2.0%	
T stage					
2	**N**	2	6	8	**0.141**
	%	4.0%	12.0%	16.0%	
3	**N**	2	35	37	
	%	4.0%	70.0%	74.0%	
4	**N**	0	5	5	
	%	.0%	10.0%	10.0%	
N stage					
0	**N**	1	9	10	**0.24**
	%	2.0%	18.0%	20.0%	
1	**N**	0	15	15	
	%	.0%	30.0%	30.0%	
2	**N**	3	14	17	
	%	6.0%	28.0%	34.0%	
3	**N**	0	8	8	
	%	.0%	16.0%	16.0%	
M stage					

0	**N**	4	34	38	**0.241**
	%	8.0%	68.0%	76.0%	
1	**N**	0	12	12	
	%	.0%	24.0%	24.0%	
Vascular invasion					
Present	**N**	1	17	18	**0.633**
	%	2.0%	34.0%	36.0%	
Absent	**N**	3	29	32	
	%	6.0%	58.0%	64.0%	
Perineural invasion					
Present	**N**	2	17	19	0.606
	%	4.0%	34.0%	38.0%	
Absent	**N**	2	29	31	
	%	4.0%	58.0%	62.0%	

*: statistically significant P value, T stage: tumor stage, N: Lymph node metastasis, M stage: Distant metastasis

E-cadherin expression was detected in all four cases associated with elevated HER2 test scores (3). **Table 4**

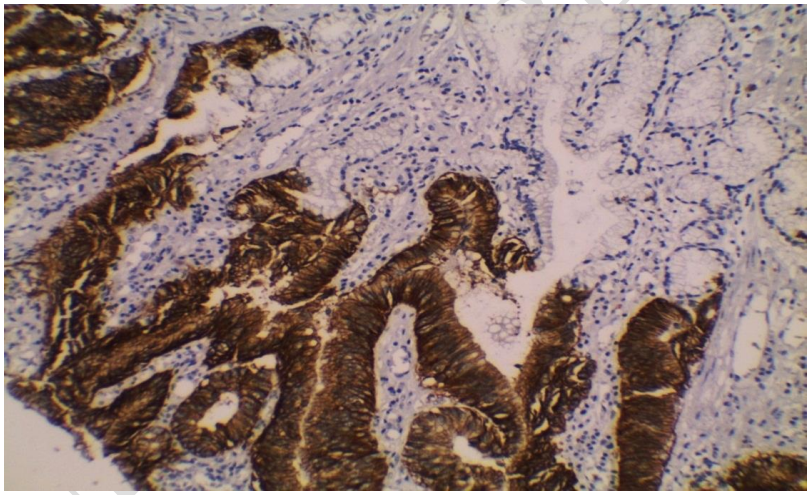

Figure 5: **Well-differentiated tubular adenocarcinoma, HER2 positive score(3), (Streptavidin- biotin x200)**

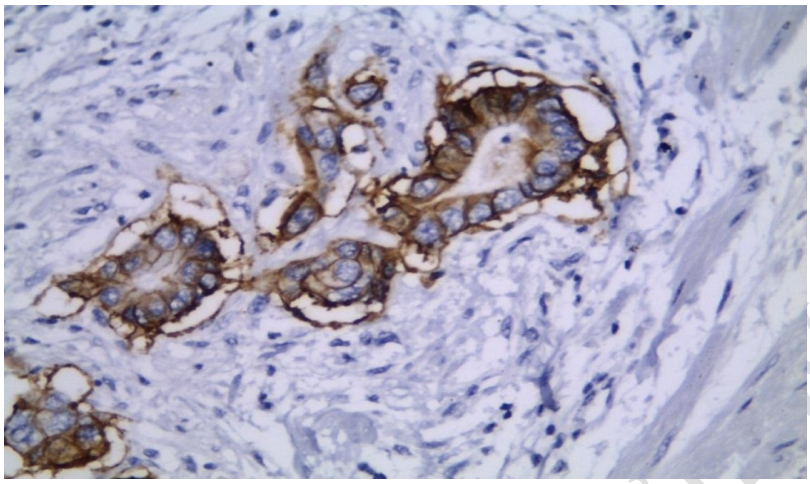

Figure 6: Well-differentiated tubular adenocarcinoma, HER2 positive score(3), showing basolateral strong membranous staining, (Streptavidin- biotin x400).

Table 4: Correlation between E-cadherin and HER2

E-cadherin		HER2		Total	P value
		+ve	-ve		
Low	N	0	19	19	0.103
	%	.0%	38.0%	38.0%	
High	N	4	27	31	
	%	8.0%	54.0%	62.0%	

Discussion

Examining the expression and prognostic significance of numerous proteins, as well as their relationship with clinicopathological features, is a critical step in developing effective targeted therapy [10].

The male to female ratio in the study was 1.5:1. These findings were also discussed in a recent paper by (Torabizadeh et al.,) [6], which included patients who were 70% male and 30% female. Saeed et al. discovered that 69.7 percent of patients were male, whereas 30.3 percent were female in their study [11]. (Sukanya et al., 2021) [8] hypothesized that men were more likely to get stomach cancer due to women's estrogen protection.

Around forty (80%) of the involved individuals were fifty years of age or older. There is substantial evidence to support the concept that old age is the primary explanation for the elderly's predominance of stomach cancer. Hui et al. (2018) [12] included 82 percent of patients above the age of 60 in their study. Additionally, Chu et al. (2020) discovered that 80% of their patients were above the age of 60 [13].

The majority of tumors were discovered in the pylorus, the stomach's bottom section, while a minority were discovered in the fundus, the body, and the cardia, the stomach's upper section. Sandeep et al. (2020) [14] discovered that the tumor was located in the distal stomach in more than half of cases. This data indicates that, whereas around 37% of cases are located in the proximal stomach, nearly 40% of cases are located in the distal stomach. Alshahrani et al. (2020) [5] discovered that the most common tumor location was the esophageal junction, which occurred in 32.2 percent of patients, while the stomach fundus occurred in just 9.3 percent of patients (11.9%). This disparity in outcomes could be explained by differences in food habits and environmental influences (Alshahrani et al., 2020) [5].

Tubular adenocarcinoma (22 of the 50 cases), papillary adenocarcinoma (3 of the 50 cases), poorly cohesive carcinoma (2 of the 50 cases, including signet ring carcinoma), mucinous adenocarcinoma (3 of the 50 cases), mixed adenocarcinoma (2 of the 50 cases), and carcinoma with lymphoid stroma were used to describe these cases histologically (1 of the 50 cases). This was consistent with a study conducted by Gulten et al (2020) [15]. Around 47% of cases were tubular adenocarcinoma, 5% papillary adenocarcinoma, 35% poorly cohesive carcinoma, 10% mucinous adenocarcinoma, 3% mixed adenocarcinoma, and 4% undifferentiated cancer.

Of the sixteen (16%) patients who underwent staging, eight (16%) had T2 staging, which indicates that the tumor has invaded the muscularis propria or subserosa, 37 (74%) had T3 staging, and five (10%) had T4 staging. Ten instances (20%) had a nodal state of N0, fifteen (30%) had a nodal status of N1, seventeen (34%) had a nodal status of N2, and only eight (20%) had a nodal status of N3 (16 percent). According to Oliveira et al. (2019) [16], penetration occurred to a depth of T1 (4.2%) in the studies, T2 (23.6%) in the studies, T3 (56.9%) in the studies, and T4 (100%) in the case studies (13.9 percent). Around 65.3 percent of individuals had lymphatic metastases. According to another study (Zhong et al.,

2021) [17], 8.5% of patients were in the T1 stage, 12.4% were in the T2, 40.6 percent were in the T3, and 38.5 percent were in the T4. N0 metastases were detected in 27.1 percent of cases, N1 metastases were detected in 26.5 percent of cases, N2 metastases were detected in 24.9 percent of cases, and N3 metastases were detected in 21.5 percent of cases.

Our analysis revealed metastases in 12 of the instances (24 percent). There were 18 instances of vascular invasion discovered (36 percent). Nearly two-thirds of patients had perineural invasion (28 percent). As can be seen from the statistics above, roughly 35.5 percent of patients in the Abd El Salam et al. (2018) [1] research had lymphovascular invasion, whereas approximately 18.4 percent had perineural invasion. Satala et al. (2020) [18] reported that roughly 18.2% of their patients had distant metastases.

Our study assessed the levels of E-cadherin and HER2 immunohistochemistry and their relationship to the clinicopathological factors we chose.

On chromosome 16, CDH1, the CDH1 gene, and the CDH1 protein are all present. (Question 22.1). Gastric epithelial cells rely on E-role cadherins for adhesion and differentiation as a protective strategy against neoplastic transformation. E-cadherin is extensively expressed in well-differentiated carcinomas, and cellular adhesion is strong, with just a minor invasive potential. However, E-cadherin expression is decreased, and intercellular adhesion is lost in poorly differentiated tumors, hence enhancing the invasive potential [19].

In our investigation, E-cadherin immunostaining was observed in 62 percent of the 31 instances with membrane staining, with an upper score of 6-12, and in 38 percent of the 19 cases with membrane staining, with a lower score of 1-4. (Irregular expression). In a study conducted by Dobritoiu et al., [20] 62% of the samples analyzed had an E-cadherin immunoreaction (2019). Rossi et al., 2019 [21] discovered that 42.2 percent of individuals with stomach cancer had abnormal E-cadherin expression (as reported by Rossi et al., 2019 [21]). Antibodies with varying specificities may produce variable results. You may observe that the findings vary depending on the number of patients included in the experiment.

Prior to this study, we noticed no correlation between tumor location and abnormal E-cadherin expression, which Piccolo et al. (2020) [22] also detected.

According to the WHO, there is a high link between E-cadherin expression and histologic changes and stages of gastric cancer. Low-grade variations had a significantly higher relative prevalence of low-grade variants (such as well-differentiated tubular adenocarcinoma and well-differentiated papillary carcinoma); moderately differentiated (8%) and tubular adenocarcinoma were much more prevalent in low-grade variants (6 percent). E-cadherin expression is low in well-differentiated high-grade tumors such signet ring carcinoma and low-grade malignant tumors like carcinoma in situ and low-grade squamous carcinoma.

This study corroborated the findings of a 2017 study published in the journal Histopathology, which demonstrated a substantial correlation E-cadherin expression and histologic type and grade. In other words, well-differentiated and moderately differentiated carcinomas expressed at a high level (27.1% and 17.1%, respectively), whereas poorly differentiated carcinomas expressed at a low level (7.1 percent). These authors also reported on the findings of Hamed et al., (2019) [23], and Kumar et al., [24] in a separate study. Inactivation of E-cadherin may result in tumor cell separation due to the loss of intercellular adhesion.

E-cadherin expression shows no statistically significant relationship with the invasion depth observed in the current study. 74 percent of patients studied were in the T3 stage, and 40% had low E-cadherin expression. Around one-fifth of cases are diagnosed at the T2 stage, with another quarter having low levels of E-cadherin. Additionally, roughly one-quarter of cases are diagnosed at the T4 stage, with 40% of them expressing low levels of E-cadherin. In agreement with Anbiaee et al., [25] 54.7% of their cases were advanced T stage and 54.5 percent had aberrantly low E cadherin expression (2013). Schizas et al. (2017) [19] and Kumar et al. (2021) [24] also confirmed similar findings. Torabizadeh et al. (2017) [6] conducted another study on E-cadherin expression and tumor stage.

In 75% of patients with distant metastases, E-cadherin expression was shown to be abnormal. Additionally, there was a high correlation between aberrant low expression and vascular invasion, as 66.6 percent of patients displayed aberrant low expression. Schizas et al., (2017) [19] and Torabizadeh et al., (2017) [6] observed no significant relationships between abnormal E-cadherin expression and distant metastases. Several previous studies have discovered significant links between decreased E-cadherin expression and metastasis

(as in studies conducted by Saad et al., 2010 [26], and Chu et al., (2008) [27]. These differences are most likely the result of patients receiving insufficient follow-up time (Torabizadeh et al., 2017) [6].

Variations in TNM staging may be due to variances in ethnic populations, a small number of patients investigated, or different E-cadherin testing procedures. To be clear, these findings underscore the intricacy of gastric carcinogenesis, which may be triggered by a variety of different etiologies, including genetic defects in a variety of genes or pathways. Larger studies are necessary to evaluate several routes in a single or multiple trials [23].

The HER2 proto-oncogene codes for a transmembrane tyrosine kinase receptor that is located on the long arm of the 17th chromosome. According to some scientists, HER2 has a key role in tumor formation because it affects cell proliferation, apoptosis, adhesion, migration, angiogenesis, and differentiation.

Four (8%) of the 50 individuals analyzed had a positive HER2 score, while 46 (92%) had a negative HER2 score (0). In a 2017 study co-authored by Ishaky et al., (2017) [28] three out of thirty (10%) HER2-positive breast cancer cases were detected. Motoshima et al. discovered Her2 in 14.5 percent of the patients they investigated. In sum, 56 percent (n=38) of the 68 individuals investigated by Roy et al., (2019) [29] had HER2 positive test findings. In a separate analysis, Panigrahi et al. (2020) [30] discovered that 26.92 percent (21 cases) were positive for Her2, 16.67 percent (13 cases) were equivocal, and 56.41 percent (44 cases) were negative.

Overproduction of HER2 occurs in the majority of stomach carcinomas. Additionally, the heterogeneity of HER2 within tumors in gastric cancer may contribute to this. The ultimate result may be influenced by the primary antibody selected (monoclonal or polyclonal). Third, the pathologist's knowledge, the grading system used, and visual perception all play a role in HER2 assessment (Sukyna et al.,2021) [8].

Her2 expression was detected in 6.6 percent of male patients and 10% of female patients in the current study but had no statistical significance. Phan et al. (2017) [31] discovered that 26.6 percent of male and 20.3 percent of female patients have HER2. Roy et al. (2019) [29] confirmed that Her2 was positive in 66% of males and 36% of females with no statistical significance. Positive incidences accounted for 5% of persons above the age of 50

in our study. 27.2 percent of their patients aged 60 or older who tested positive for Her2 had no clinical significance. This study may account for the greater incidence of stomach cancer in males and the elderly.

One of the 17 cases (11.8%) identified in the cardiac zone, one case (20%) out of ten cases (20%) in the fundus, and one case (4.2%) out of 24 cases (4.2%) in the El-Gendi et al., (2015) [32] all concurred (2020).

HER2 was positive in one case (25 percent of well-differentiated tubular cancer cases) and in three cases (30 percent of moderately differentiated tubular adenocarcinoma cases) with a positive score of 3. However, HER2 was found to be negative in six of the remaining instances (0). When it was observed that 47.6 percent of cases of moderately differentiated adenocarcinoma were HER2 3+ positive, 36.8 percent of cases of well-differentiated adenocarcinoma were HER2 3+ positive. Our findings differed with those of Madani et al., (2015) [33], who reported that moderately and well-differentiated cases (17.5%) were the most prevalent subtype of HER2-positive cases (11.7%). These findings could be the result of a variety of factors, including different sample sizes and case selection.

Of N0 and N2 cases 10% and 3%, respectively, were positive for HER2 (3). Abd el Salam et al. (2018) [1] discovered that HER2 was positive in nearly half of all patients with N0, 55.2 percent of cases with N1, 55.2 percent of cases with N3, and 60% of cases with N4. This investigation determined that there was no statistical significance. Mohapatra et al. (2020) [34] confirmed our findings, reporting that 47.22 percent of patients with lymph node metastases exhibited substantial Her2 positivity. This could be because the cases were identified later in the disease's course when it may have been too late to intervene.

This study discovered that HER2 was present in around 10.5 percent of cases without distant metastasis but was negative in all cases with distant metastasis. This was consistent with Satala et al (2020).'s [18] finding that 11.8 percent of cases without distant metastasis were HER2-positive, and 11.7 percent of those with distant metastasis were HER2-positive (3). Motoshima et al. (2018) [35] detected this in 44% of cases with distant metastases. One reason for the discrepancies is that they are due to differences in the presentation and quantity of cases analyzed between researches.

In terms of vascular invasion, 5.5 percent of patients demonstrated signs of vascular emboli, while 9.4 percent did not. In a study, positive was detected in 24.6 percent of individuals with vascular emboli and 24.5 percent of cases without vascular emboli. In contrast to what Abd El Salam et al. (2018) [1] discovered, only 15% of individuals with vascular emboli tested positive for HER2.

Three of the four patients with HER2 expression had normal E-cadherin expression. Kandel et al. (2016) [36] similarly observed a high (normal) level of E-cadherin expression in her2-positive tumor tissues. While it is believed that overexpression of HER2 promotes tumor cell survival and proliferation, E-activity cadherin acts as an anti-invasive protein on the epithelial membrane, limiting tumor growth. If we had to make a hypothesis, we may suggest that HER2 amplification serves as a substitute for E-cadherin expression in the creation of tumors, having a higher effect on tumor progression than E-cadherin inhibition.

Conclusion

Elevated E-cadherin expression is associated with tumors that exhibit aggressive clinicopathologic characteristics. HER2 was expressed positively in low-grade variants, but no correlation was established with clinical characteristics. E-cadherin and HER2 expression have no discernible relationship.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

Comment [U2]: Reference to be arranged properly

1. Abd ElSalam A, El-Hawary A, Mohamed M, Gamil T. Immunohistochemical Expression of Her2/neu in gastric carcinomas in egyptian patients. J Clin Pathol. 2018;1:1-3.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.

3. Rotondo R, Rizzolio F, Perin T, Berretta M, Zanconati F, Giordano A, et al. Pathological Diagnosis and Classification of Gastric Epithelial Tumours. 2019. p. 53-82.
4. Tan M, Balakrishnan M, Graham D. Gastric Cancer Worldwide Except Japan: With Special Focus on Studies from Japan. 2019. p. 17-28.
5. Alshahrani S, Baabbad F, Bahobail M, Hawsawi A, Jastania E, Bamousa S, et al. Survival Time in Treatment Modalities of Gastric Carcinoma at King Khalid Hospital- Jeddah Saudi Arabia: a Retrospective Cohort Study. Mater Sociomed. 2020;32:271-6.
6. Torabizadeh Z, Nosrati A, Sajadi Saravi SN, Yazdani Charati J, Janbabai G. Evaluation of E-cadherin Expression in Gastric Cancer and Its Correlation with Clinicopathologic Parameters. Int J Hematol Oncol Stem Cell Res. 2017;11:158-64.
7. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Molecular Biology International. 2014;2014:852748.
8. Sukanya JS, Raj PV, Thanka J. Role of HER2neu expression in gastric cancer. Indian J Pathol Microbiol. 2021;64:58-64.
9. Khattak MT, Hassan M, Nasib B, Qamar MA, Javed S, Ali N. Frequency of Human Epidermal Growth Factor Receptor 2 (Her2/Neu) Expression in Gastric Adenocarcinoma in Rehman Medical Institute Peshawar. J Pak Med Assoc. 2019;69:788-93.
10. Kong X, Wang JL, Chen HM, Fang JY. Comparison of the clinicopathological characteristics of young and elderly patients with gastric carcinoma: a meta analysis. J Surg Oncol. 2012;106:346-52.
11. Saeed NA-H, Saeed A, Luma Q, Ali A, Zabbon Z, Saeed. EXPRESSION OF TUMOR SUPPRESSOR GENE P53 CORRELATION WITH GASTRIC ADENOCARCINOMA PATIENTS BY USING IMMUNOHISTOCHEMICAL ASSAY2018.
12. Hui HX, Hu ZW, Jiang C, Wu J, Gao Y, Wang XW. ZNF418 overexpression protects against gastric carcinoma and prompts a good prognosis. Onco Targets Ther. 2018;11:2763-70.
13. Chu Y, Li H, Wu D, Guo Q. HER2 protein expression correlates with Lauren classication and P53 in gastric cancer patients. Res Sq. 2020;1:203.
14. Sandeep B, Huang X, Li Y, Mao L, Gao K, Xiao Z. Gastric Carcinoma in Young Patients and Its Clinicopathological Characteristics and Prognosis. Gastroenterol Res Pract. 2020;2020:7378215.

15. Gülten G, Yilmaz B, Demirkan N. Comparing human epidermal growth factor receptor 2 amplification and expression using immunohistochemistry and silver in situ hybridisation in gastric carcinoma and lymph node metastasis. Oncol Lett. 2020;20:1897-905.
16. Oliveira LA, Oshima CTF, Soffner PA, Silva MS, Lins RR, Malinverni ACM, et al. THE CANONICAL WNT PATHWAY IN GASTRIC CARCINOMA. Arq Bras Cir Dig. 2019;32:e1414.
17. Zhong Q, Chen QY, Parisi A, Ma YB, Lin GT, Desiderio J, et al. Modified ypTNM Staging Classification for Gastric Cancer after Neoadjuvant Therapy: A Multi-Institutional Study. Oncologist. 2021;26:e99-e110.
18. Satala CB, Jung I, Stefan-van Staden RI, Kovacs Z, Molnar C, Bara T, et al. HER2 Heterogeneity in Gastric Cancer: A Comparative Study, Using Two Commercial Antibodies. Journal of Oncology. 2020;2020:8860174.
19. Schizas D, Moris D, Michalinos A, Kanavidis P, Oikonomou D, Papalampros A, et al. E-cadherin in gastric carcinomas: Relations with histological parameters and its prognostic value. J buon. 2017;22:383-9.
20. Dobriţoiu M, Stepan AE, Mărgăritescu C, Simionescu CE, Vere CC, Schenker M, et al. Immunoexpression of E-cadherin, P-cadherin and fibronectin in gastric carcinomas. Rom J Morphol Embryol. 2019;60:573-9.
21. Rossi T, Tedaldi G, Petracci E, Abou Khouzam R, Ranzani GN, Morgagni P, et al. E-cadherin Downregulation and microRNAs in Sporadic Intestinal-Type Gastric Cancer. Int J Mol Sci. 2019;20.
22. Piccolo G, Zanghì A, Di Vita M, Bisagni P, Lecchi F, Cavallaro A, et al. The role of E-cadherin expression in the treatment of western undifferentiated early gastric cancer: Can a biological factor predict lymph node metastasis? PLoS One. 2020;15:e0232429.
23. Hamed M, Fawzy G, Hanna H. The role of E-cadherin expression and E-cadherin gene promoter hypermethylation in gastric carcinoma. The Egyptian Journal of Surgery. 2019;38:361-8.
24. Kumar P, Sebastian A, Verma K, Dixit R, Kumari S, Singh J, et al. mRNA Expression Analysis of E-Cadherin, VEGF, and MMPs in Gastric Cancer: a Pilot Study. Indian J Surg Oncol. 2021;12:85-92.
25. Anbiaee R, Mojir Sheibani K, Torbati P, Jaam H. Abnormal expression of e-cadherin in gastric adenocarcinoma, and its correlation with tumor histopathology and helicobacter pylori infection. Iran Red Crescent Med J. 2013;15:218-22.

26. Saad AA, Awed NM, Abd Elkerim NN, El-Shennawy D, Alfons MA, Elserafy ME, et al. Prognostic significance of E-cadherin expression and peripheral blood micrometastasis in gastric carcinoma patients. Ann Surg Oncol. 2010;17:3059-67.
27. Chu YQ, Ye ZY, Tao HQ, Wang YY, Zhao ZS. Relationship between cell adhesion molecules expression and the biological behavior of gastric carcinoma. World J Gastroenterol. 2008;14:1990-6.
28. Ishaky E, El-Sharkawy S, Ayob M, Sharaf H, Bakeer R. Immunohistochemical expression of HER-2/neu receptors in gastric carcinoma. Journal of The Arab Society for Medical Research. 2017;12:13-8.
29. Roy PS, Nyodu T, Hazarika M, Saikia BJ, Bhuyan C, Inamdar A, et al. Prevalence of HER2 Expression and Its Correlation with Clinicopathological Parameters in Gastric or Gastroesophageal Junction Adenocarcinoma in North-East Indian Population. Asian Pac J Cancer Prev. 2019;20:1139-45.
30. Panigrahi R, Sucharita S, Rath J, Senapati U. HER2/neu expression in gastric carcinoma and its association with Helicobacter pylori infection and other clinicopathological parameters. Indian Journal of Pathology and Oncology. 2020;7:447-51.
31. Phan DAT, Nguyen VT, Hua TNH, Ngo QD, Doan TPT, Nguyen ST, et al. HER2 Status and Its Heterogeneity in Gastric Carcinoma of Vietnamese Patient. J Pathol Transl Med. 2017;51:396-402.
32. El-Gendi S, Talaat I, Abdel-Hadi M. HER-2/Neu Status in Gastric Carcinomas in a Series of Egyptian Patients and Its Relation to Ki-67 Expression. J Pathol. 2015;5:101-13.
33. Madani SH, Sadeghi E, Rezaee A, Sadeghi M, Khazaee S, Amirifard N, et al. Survey of HER2-neu Expression in Colonic Adenocarcinoma in the West of Iran. Asian Pac J Cancer Prev. 2015;16:7671-4.
34. Mohapatra D, Chakraborty K, Das D, Biswal R. Significance of HER 2/neu in gastric adenocarcinomas, a clinicopathological correlation. JMSCR 2020;8:481-7.
35. Motoshima S, Yonemoto K, Kamei H, Morita M, Yamaguchi R. Prognostic implications of HER2 heterogeneity in gastric cancer. Oncotarget. 2018;9:9262-72.
36. Kandel C, Leclair F, Bou-Hanna C, Laboisse CL, Mosnier JF. Association of HER1 amplification with poor prognosis in well differentiated gastric carcinomas. J Clin Pathol. 2014;67:307-12.