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Immunohistochemical expression of E-Cadherin in gastric carcinoma in Erbil city, Kurdistan Region

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Abstract

Background and objectives: Gastric carcinoma is at fourth position incidence-wise and holds a second place for deaths due to cancer. The Cadherins represent an important class of the adhesiveness molecules, which play an essential role in the homotypical cell-cell adhesion and in the complex process of invasion and metastasis. The point of this study is to identify the rate of the E-Cadherin immunoexpression in gastric carcinoma in Erbil locality and investigate its association with the clinico pathological parameters.

Methods: A retrospective study was carried out in Rizgary teaching hospital and private laboratories in Erbil city for the period from Jan.2018 - Jan.2020. A total of 75 formalin fixed, paraffin embedded archival tissue blocks of total or partial gastrectomy samples for gastric carcinoma cases were collected, then E-cadherin immunohistochemistry was tested.

Results: Twenty-one cases (28%) were categorized as high expression for E-Cadherin immunoexpression. At the same time 38 (50.7%) of the cases were classified as low expression and 16 (21.3%) of cases were labelled with no expression. E-Cadherin status was significantly associated with the tumor type and tumor grade, in which high E-Cadherin expression about (61.9%) was demonstrated in intestinal type tumors, (38.1%) in diffuse type tumors and about (57.1%) was performed in well-moderately differentiated tumors and (42.9%) in poorly differentiated tumors. While no significant association was found between E-Cadherin expression and other clinico pathological parameters

Conclusions: Our result support the hypothesis that the E-Cadherin immunostain is commonly expressed in Gastric cancer, and it was significantly related with type and grade of the tumor.

Key words: Gastric carcinoma, E-Cadherin; Immunoexpression.

Introduction

Gastric carcinoma is at fourth position incidence-wise and holds second place for deaths due to cancer. The disease commonly has a bad prognosis and Western journalism reported only 30% five-year survival rates1-2. Based on Lauren's system, gastric carcinomas have been divided histologically into intestinal and diffuse type tumors. The American Joint Committee on Cancer (AJCC) accepts this classification system as it offers correlation between histological types and epidemiological data3-4. Gastric carcinoma results from interaction between genetic and environmental factors. H. pylori infection has appeared as a high risk factor for this cancer5 Helicobacter pylori(H.

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pylori) positive patients are considered to have a two- to three-fold increased risk of evolving gastric cancer when compared with H. pylori-negative patients 6-9. Infection with H. pylori leads to complete or partial loss of E-cadherin and is believed to be associated with early occurrences in gastric cancer10-11. Recently researchers have discovered that H. pylori secretes a protease enzyme called HtrA used like a weapon to penetrate. This enzyme splits the three proteins occludin, claudin-8 and E-cadherin, breaking the layer of epithelial cells. As a result, H. pylori bacteria can get deeper and inflict further damage. This is the first step concerning gastric cancer starting to progress12. Cadherin is a superfamily of calcium-mediated membrane glycoproteins, with a molecular mass of 120 ku, forming one of the four classes of adhesion molecules13-14. E-cadherin which is a chief cell adhesion molecule is a glycoprotein located in the cell membranes and is regarded as a tumor suppressor gene. It is assumed to have a role in suppression of invasion by gastric carcinoma15-16. Loss of CDH1 gene that is responsible for E-cadherin expression is seen often in diffuse-type gastric cancers many of which are hereditary17-18.Many different cancers including gastric cancers have aberrant E-cadherin expression. As the E-cadherin expression reduces, the tumor cell cohesiveness will be reduced, thereby favoring metastasis19-20. Some common cadherins expressed by epithelial cells are belong to E-cadherin, N-cadherin and P-cadherin. The intracellular domains of classical cadherins interact with β-catenin and γ-catenin to assemble the cytoplasmic cell adhesion complex (CCC) that is critical for the formation of extracellular cell-cell adhesion. β-catenin and γ-catenin bind directly to α-catenin, which links the CCC to the actin cytoskeleton21-22. The aim of this study was to detect the frequency of the E-Cadherin immunoexpression in gastric carcinoma and investigate its association with some clinicopathological parameters

Material and methods

A cross-sectional study was conducted after permission approval granted by Kurdistan board for medical specialty. A total of 75 gastrectomy specimens paraffin blocks retrieved from Histopathology laboratory of Rizgary Teaching Hospital and some private histopathology labs in Erbil city were used in this study.Tissue samples of gastrectomy specimen stained with H&E were histologically examined. Gastric carcinomas were labeled as intestinal or diffuse type. Histological grade was coded as well - moderately differentiated, and poorly differentiated. The pathological tumor staging was performed according to AJCC and the Union Internationale Contre Le Cancer (UICC), by grouping the various TNM components23. We assess E-Cadherin expression in gastric cancer cases using immunohistochemical method. Positive control was a normal gastric mucosa with a strong membranous staining for E-cadherin. Negative controls were prepared simultaneously for all 75 samples by replacing the primary antibody with distilled water. Dako Manufacturer's recommendations were followed for immunostaining (DakoEnVision™ Flex). The tissue was stained by Labeled polymer and enhanced polymer systems (Autostainer Link 48) method. Thin sections (four μm) were cut, mounted on salinized slides, and dried at 60 °C for one hour. Tissue slides were then deparaffinized and rehydrated at

room temperature (20-25 °C). Slides were placed in a xylene bath and incubated for five minutes and then put in absolute ethanol for 3 minutes. After that, slides were placed in 95% ethanol for 3 minutes. Finally, slides were immersed in distilled or deionized water for a minimum of 30 seconds. A specific epitope retrieval method in 10 mmol/L citrate buffer was used using 1:10 ratio with distilled water. Two expert pathologists had examined the sections with the use of light microscopy independently. Any disagreements were reviewed and followed by definitive decisions. E-cadherin expression was evaluated semi quantitatively and then scored according to the percentage of labeled cells. Staining intensity of E-Cadherin was reported on a scale with three grades: Positive membranous staining in less than 10% of tumor cells: 0, Positive membranous staining between 10% to 90% of tumor cells: +1, Positive membranous staining Over 90% of the tumor cells: +2, 0 grade as No E-Cadherin expression and 1+ grade as low expression while +2 regarded as high expression24. Statistical analysis was performed by SPSS program version 23; level of significance will be set at ($p \leq 0.05$). The chi square test will be used to assess the association between E-Cadherin expression and clinicopathological parameters.

Results

From total of 75 patients, 46 were males and 29 were females, with male: female ratio of 1.55:1, aged between 26-90 years with a mean age 61.21, the median age was 62 years.Analysis of E-Cadherin expression for 75 studied gastric carcinomas showed immunoreactivity with variable index of No expression(scoring 0) in 16(21.3%) cases, in which 3(18.8%) of them were of intestinal type and 13(81.3%) were of diffuse type since low expression(scoring1+) in 38(50.7%) cases, in which 28(73.7%) of them were of intestinal type and 10(26.3%) of them were of diffuse type, while high E-Cadherin expression (score 2+) in 21(28%) cases composed of 13(61.9%) intestinal types and 8(38.1%) diffuse types as shown in Figure (1).

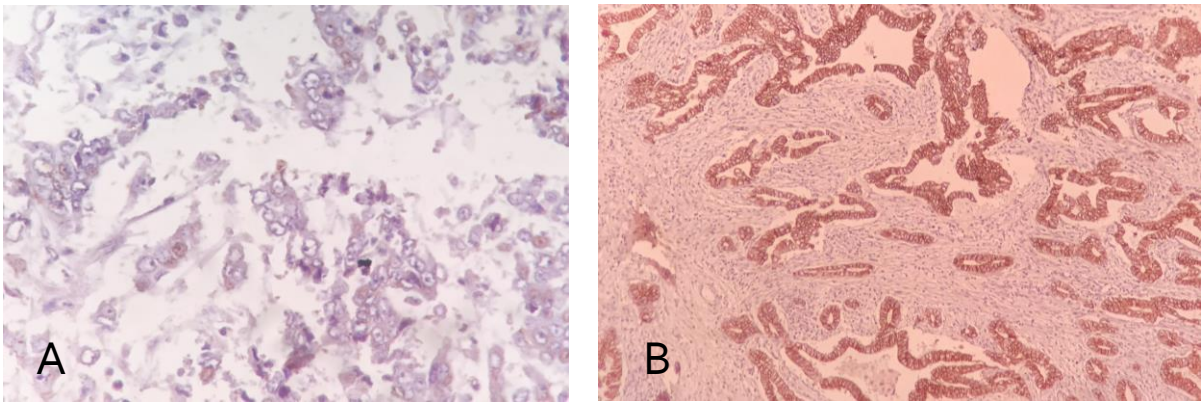

Figure (1): E-cadherin immunoexpression score 0 (IHCX400) (A), and score 2+ (IHCX400) (B).

E-Cadherin status was significantly associated with tumor type (p= 0.001) and tumor grade (p=0.03) while its association with other clinicopathological parameters had no significance, as shown in Table (1).

Table (1): association of E-Cadherin expression and clinicopathological parameters.

Variables	Categories	E-cadherin expression			Total	P - value
		No expression	Low expression	High expression		
Gender	Male	10 (62.5%)	24 (63.2%)	12 (57.1%)	46 (61.3%)	0.89
	Female	6 (37.5%)	14 (36.8%)	9 (42.9%)	29 (38.7%)	
Tumor Type	Intestinal type	3 (18.8%)	28 (73.7%)	13 (61.9%)	44 (58.7%)	0.001
	Diffuse type	13 (81.3%)	10 (26.3%)	8 (38.1%)	31 (41.3%)	
Tumor grade	Well-moderate differentiated	5 (31.3%)	9 (23.7%)	12 (57.1%)	26 (34.7%)	0.03
	Poorly differentiated	11 (68.8%)	29 (76.3%)	9 (42.9%)	49 (65.3%)	
Lymphovascular invasion	Positive	12 (75%)	29 (76.3%)	19 (90.5%)	60 (80%)	0.38
	Negative	4 (25%)	9 (23.7%)	2 (9.5%)	15 (20%)	
Nodal status	Positive	12 (75%)	33 (86.8%)	20 (95.2%)	65 (86.7%)	0.21
	Negative	4 (25%)	5 (13.2%)	1 (4.8%)	10 (13.3%)	
Age groups	≤ 50 years	4 (25%)	7 (18.4%)	6 (28.6%)	17 (22.7%)	0.67
	> 50 years	12 (75%)	31 (81.6%)	15 (71.4%)	58 (77.3%)	
Tumor stage	1-2	8 (50%)	8 (21.1%)	6 (28.6%)	22 (29.3%)	0.103
	3- 4	8 (50%)	30 (78.9%)	15 (71.4%)	53 (70.7%)	
Total		16 (100%)	38 (100%)	21 (100%)	75 (100%)	

Discussion

The Cadherins represent an important class of the adhesiveness molecules, which play an essential role in the homotypical cell-cell adhesion and in the complex process of invasion and metastasis[25.]Moreover, the loss of the cellular adhesiveness may contribute to the contact inhibition disappearance, this may play an important role in the first stage of carcinogenesis[26-28]. Comparable results were found in previous studies regarding patient gender that show E-cadhrin expression was more frequent between males than females (61.3% versus 38.7%) with no statistically significant association[29.] In the present study, high E-cadherin expressions were highly collective among older age groups (age > 50 years), still there was no statistically significant association between them, which was also compatible with other study[29.] The majority of high expressed E-cadherin found in intestinal type (58.7%) than in diffuse type (41.3%) with statistically significant association between them in which high expression found in (61.9%) and (38.1%) in intestinal and diffuse type tumors respectively, low expression in (73.7%) for the former and (26.3%) for the latter and there was no expression in (18.8%) of intestinal types and (81.3%) in diffuse types (p=0.001). This result was in agreement with other studies that found E-cadherin expression more expressed in intestinal types rather than in diffuse types of gastric carcinomas[30-31], while another study discovered that E-cadherin immunohistochemical expression have been noticed significantly more commonly in diffuse type carcinomas (82.4%) in comparison to intestinal types (31.6%)[29]. There is also statistically significant association was found between high E-cadherin expression and tumor grade, in which high expression about (57.1%) in well-moderately differentiated cases and (42.9%) of poorly differentiated cases while low E-cadherin expression was higher

among poorly differentiated cases which was about(76.3%) and about(23.7%) in well-moderate differentiated cases, and there was no expression in (31.3%) in well-moderate differentiated tumors and (68.8%) in poorly differentiated cases (p=0.03). Similar results were found in other study[32] in which significant association was found between E-cadherin expression and tumor grade; on the other hand, an opposite result was found in other study [29], where E-cadherin immunohistochemical expression was noted more often in poorly differentiated carcinomas (61.5%) in comparison to moderately differentiated carcinomas (30%), also in agreement with our study the association was significant.Our study shows that the rate of high E-cadherin expression in cases with lymph node metastasis was (95.2%) which was extremely greater than those with no lymph node metastasis (4.8%), also in tumors with lymphovascular invasion had much more higher E-cadherin immunoexpression (90.5%) than those with no lymphovascular invasion (9.5%) in contrast to previous studies showed that tumors with lymphovascular invasion had no E-Cadherin expression[29], there was no statistically significant association with both clinicopathological parameters. Concerning the association of E-cadherin expression with the stage of the tumor, although statistically non-significant, E-cadherin highly expressed in higher stages (III, IV) (71.4%) than in lower stages (I, II) (28.6%). The wide range of E-cadherin expression and differences in association with clinicopathological parameters among different studies might be because of variable issues including sample size and immunohistochemichal procedure, type of antibody, method of antigen retrieval and scoring systems.

Conclusions

E-cadherin was commonly expressed in gastric carcinomas and it was significantly associated with tumor type and tumor grade, while there was no significant association with other clinicopathological parameters.

Conflict of interests

There were no conflicts of interest.

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