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Original article

Significance of Ephrin Type-B Receptor 2 (EphB2) and Cell Adhesion Molecule 4 (CADM4) Expression in Gastric Carcinoma: an Immunohistochemical Study

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Abstract:

Background: Globally, Gastric carcinoma (GC) is one of the most common cancers and leading causes of cancer death. The role of Ephrin type-B receptor 2 (EphB2) and Cell adhesion molecule 4 (CADM4) in initiation or progression of cancers was assessed in multiple studies. However, there is a debate about their role in GC. **Aim:** To evaluate EphB2 and CADM4 immunohistochemical expression in GC and its precursor lesions to assess their possible roles. **Material and method:** In this retrospective study, EphB2 and CADM4 immunostaining was performed for 50 selected cases of GC, 10 cases of chronic gastritis, 6 cases of chronic gastritis with intestinal metaplasia and 6 cases of gastric adenoma. **Results:** There was a highly significant statistical difference between the study groups regarding EphB2 and CADM4 expression (P= 0.002 and < 0.001 respectively). EphB2 expression was significantly associated with increased depth of tumor invasion, lymph node metastasis, distant metastasis and advanced stage (P= 0.006, 0.026, 0.016 and < 0.001 respectively). Loss of CADM4 expression was significantly associated with certain histopathological subtypes, high grade tumors, distant metastasis and advanced stage (P= 0.032, 0.015, 0.007 and 0.012 respectively). Low CADM4 expression was significantly associated with increased depth of tumor invasion, distant metastasis and advanced stage (P= 0.027, 0.006 and 0.003 respectively). **Conclusion:** EphB2 and CADM4 may have a role in pathogenesis and progression of GC and may work combined as useful prognostic markers and therapeutic targets for GC patients.

Keywords: Gastric carcinoma, EphB2, CADM4.

Abbreviations: Gastric carcinoma (GC), Ephrin type-B receptor 2 (EphB2), Cell adhesion molecule 4 (CADM4).

Introduction

The fourth most common cause of cancer-related mortality and the fifth most prevalent cancer worldwide in 2020 is gastric cancer. Eastern Europe and Asia have high incidence rates, whereas Northern Europe and America have low rates that are parallel to those seen across Africa [1].

The GLOBOCAN 2022 statistics show that gastric cancer accounts for 2.2% of all cancers in Egypt, placing it in the eleventh rank [2]. At a median age of 53 years, gastric cancer accounted for 1.8% of all malignancies and 10.3% of gastrointestinal cancers, according to the National Cancer Institute (NCI) in Cairo [3].

Gastric carcinogenesis is a multi-step process, characterized by a complex interaction between environmental and host factors. The chronic infection with Helicobacter pylori (H. pylori) is the most important factor among them [4].

Surgery, radiation, neoadjuvant chemotherapy, and immunotherapy are the current methods for GC treatment. Patients diagnosed with early GC have a survival rate of about 90%. However, the survival rates are reduced due to difficulty in diagnosing this cancer at an early stage [5].

Gastric carcinomas often acquire phenotypic and genetic alterations that render them resistant to conventional therapy. This emphasizes the importance of identifying diagnostic and prognostic markers, as well as therapeutic targets, for GC patients [6].

Ephrin type-B receptor 2 (EphB2) belongs to the Eph receptor family and the intestinal stem cell signature genes. It is essential for the regulation of cell migration and organization of the cytoskeleton. Its involvement in carcinogenesis is a matter of debate, particularly in GC [7].

Cell adhesion molecule 4 (CADM4) is a member of the immunoglobulin superfamily of cell adhesion molecules and is essential for suppression of tumors. CADM4 is primarily expressed in the brain, bladder, prostate, and kidney and its expression was studied in many cancers such as breast, prostate, colon and lung cancer [8].

The aim of this work is to assess the significance of immunohistochemical expression of EphB2 and CADM4 in GC and precursor lesions.

Material and methods:

Study Groups:

This is a retrospective study included 50 selected cases of GC and 22 cases of non neoplastic and precursor lesions consisting of 10 cases of chronic gastritis, 6 cases of chronic gastritis with intestinal metaplasia and 6 cases of gastric adenoma with low grade dysplasia. The archived patient files were the source of the clinical data.

Archival formalin fixed paraffin embedded blocks processed between 2017 and 2023 were included in the material. These blocks were obtained from the Early Cancer Detection Unit and Pathology Department; Faculty of Medicine, Benha University. Because of the retrospective nature of the study, a documented informed consent was not necessary. The Research Ethical committee of Faculty of Medicine, Benha University granted the approval of this study (MD 18-8-2022).

Inclusion criteria:

Cases with available demographic and clinical records and available blocks. The GC cases were surgically treated by subtotal or total gastrectomy in Benha university hospitals during the years 2017-2023.

Exclusion criteria:

Cases with preoperative neoadjuvant therapy, cases with unavailable demographic and clinical records and GC cases diagnosed only by endoscopic biopsies without further surgical management.

Histopathological studies:

Sections from all cases were examined without regard to their diagnosis. In accordance with the WHO classification, 5th edition, The GC cases were evaluated for their subtype and classified as low-

grade tumors (well and moderately differentiated) and high-grade tumors (poorly differentiated) [9]. In accordance with the TNM staging system, GC cases were categorized into stage I, II, III, IV. Statistical analysis was conducted by combining stage I and stage II cases [10].

Immunohistochemical studies:

EphB2 and CADM4 antibody staining was performed for all cases, using Avidin-Biotin complex technique, in accordance with the manufacturer's instructions.

Positive control:

A section of normal colon was used as external positive control for EphB2 [11] and normal renal tissue for CADM4 [12].

Negative control:

Negative control was done by replacing the primary antibody in the run with Phosphate Buffered Saline (PBS).

Normal control:

As a control group, 6 cases of apparently normal gastric tissue were taken from patients who underwent bariatric surgery.

Immunohistochemical assessment:

The presence of cytoplasmic or membranous staining was interpreted as a positive for EphB2 expression. Using a semi-quantitative scoring method, the intensity of EphB2 expression scores was evaluated (0: negative; 1: weak; 2: moderate; 3: strong). The percentage of the staining was scored (0: none, 1: 1% to 33%; 2: 34% to 66%; 3: 67% to 100%). The two scores were added. The total scores of 0–2 were interpreted as negative and 3–6 as positive [13, 14].

Cytoplasmic CADM4 expression was evaluated using an established method measuring the intensity and extent of staining. The intensity was scored as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The extent was as follows: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%). Intensity and extent were multiplied to get the final scores (0–12). Scores of 3 to 12 were positive and scores of 0 to 2 were negative for CADM4 expression. The positive scores were divided into low expression group (score 3–7) and high expression group (score 8–12) to facilitate statistical analysis [15, 16].

Statistical analysis:

Mean ± standard deviation (SD) was used to express quantitative data, whilst categorical data were presented as number and percentages and analyzed using Fisher's exact test or Chi square test (χ^2). Receiver-operating characteristic (ROC) curve was used to detect validity of EphB2 and CADM4 expression in GC. P values less than 0.05 were significant, whereas P values more than or equal to 0.01 were highly significant. The SPSS version 22 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results:

Demographic findings and clinico-pathological results:

The mean age of studied cases was 58±12.7 with their age ranging between 37 and 81 years. Twenty-four cases (48%) aged < 58 years and 26 cases (52%) aged ≥ 58 years. Twenty-six cases (52%) were males, while 24 cases (48%) were females **table (1).**

Immunohistochemical results:

EphB2 results

In the different study groups, EphB2 expression showed a highly significant statistical difference (P value = 0.002). EphB2 expression was negative in the normal control group **(table 2, figure 1, 2).**

The relation between EphB2 expression in studied GC cases and clinicopathological variables was statistically analyzed. It revealed that positive EphB2 expression was significantly associated with increased depth of tumor invasion (P-value = 0.006), lymph node metastasis (P-value = 0.026), distant metastasis (P-value = 0.016) and high TNM stage (P-value < 0.001) **(table 3).**

CADM4 results

In the different study groups, CADM4 expression showed a highly significant statistical difference (*P* value <0.001).

CADM4 expression was positive in the normal control group **(table 2, figure 1, 2).**

The relation between CADM4 expression in studied GC cases and clinicopathological variables was statistically analyzed. It revealed that negative CADM4 expression was significantly associated with certain histopathological subtypes (poorly cohesive carcinoma and mucinous adenocarcinoma), high grade tumors, distant metastasis and advanced stage (P= 0.032, 0.015, 0.007 and 0.012 respectively). **(table 3).** It also revealed that low CADM4 expression was significantly associated with increased depth of tumor invasion, distant metastasis and advanced stage (P= 0.027, 0.006 and 0.003 respectively) **(table 4).** There was statistically insignificant relation between EphB2 expression and CADM4 expression in studied GC cases (P-value = 0.201) **(table 5).**

ROC curve analysis revealed that EphB2 is more sensitive than specific, while CADM4 is more specific than sensitive in predicting GC from non neoplastic and precursor lesions as well as in predicting gastric dysplasia and carcinoma from non dysplastic lesions **(table 6 and figure 3).**

Table (1) Clinico-pathological results of studied gastric carcinoma cases

Histopathological subtype	
Tubular adenocarcinoma	32 (64%)
Poorly cohesive carcinoma	12 (24%)
Mucinous adenocarcinoma	6 (12%)
Tumor grade	
Low grade	29 (58 %)
High grade	21 (42 %)
Depth of tumor invasion (T)	
T1	6 (12%)
T2	6 (12%)
T3	16 (32%)
T4	22 (44%)
Lymph node metastasis (N)	
N0	7 (14%)
N1	8 (16%)
N2	20 (40%)
N3	15 (30%)
Distant metastasis (M)	
M0	30 (60%)
M1	20 (40%)
TNM stage	
Stage I	2 (4%)
Stage II	12 (24%)
Stage III	16 (32%)
Stage IV	20 (40%)

Table 2: Comparison between the different study groups regarding EphB2 and CADM4 expression.

Study groups	EphB2 expression		P-value	CADM4 expression			
	negative	positive		negative	positive		
					Low expression	High expression	P-value
Chronic gastritis (N=10)	8 (80%)	2 (20%)	0.002**	0	0	10 (100%)	
Chronic gastritis with intestinal metaplasia(N=6)	4 (66.7%)	2 (33.3%)		1 (16.7%)	5 (83.3%)	0	
Gastric dysplasia (N=6)	2 (33.3%)	4 (66.7%)		2 (33.3%)	4 (66.7%)	0	<0.001**
Gastric carcinoma (N=50)	12 (24%)	38 (76%)		21 (42%)	11 (22%)	18 (36%)	
Total (N=72)	26(36.1%)	46(63.9%)		24(33.3%)	20(27.8%)	28(38.9%)	

EphB2, Ephrin type B receptor 2; N, number; ** highly significant.

Table 3: Comparison between EphB2 and CADM4 expression in studied cases according to clinico-pathological features:

Clinico-pathological features		Total	EphB2 expression		P-value	CADM4 expression		P-value
			Negative	Positive		Negative	Positive	
Tumor subtype	Tubular adenocarcinoma	32	10(32.2%)	22(68.8%)	0.344	9(28.1%)	23(71.9%)	0.032*
	Poorly cohesive carcinoma	12	1 (8.3%)	11(91.7%)		8(66.7%)	4 (33.3%)	
	Mucinous adenocarcinoma	6	1 (16.7%)	5 (83.3%)		4(66.7%)	2 (33.3%)	
Tumor grade	Low grade	29	9 (31%)	20 (69%)	0.201	8(27.6%)	21(72.4%)	0.021*
	High grade	21	3 (14.3%)	18(85.7%)		13(61.9%)	8 (38.1%)	
Depth of tumor invasion (T)	T1	6	4 (66.7%)	2 (33.3%)	0.006**	0	6 (100%)	0.149
	T2	6	2 (33.3%)	4 (66.7%)		3 (50%)	3 (50 %)	
	T3	16	5 (31.3%)	11(68.7%)		7(43.8%)	9 (56.2%)	
	T4	22	1 (4.5%)	21(95.5%)		11 (50%)	11 (50%)	
Lymph node metastasis (N)	N0	7	3 (42.9%)	4 (57.1%)	0.026*	3 (42.9%)	4 (57.1%)	0.196
	N1	8	3 (37.5%)	5 (62.5%)		5 (25%)	15 (75%)	
	N2	20	6 (30%)	14 (70%)		9 (60%)	6 (40%)	
	N3	15	0	15 (100%)		0	15 (100%)	
Distant metastasis (M)	M0	30	11(36.7%)	19(63.3%)	0.016*	8 (26.7%)	22(73.3%)	0.01*
	M1	20	1 (5%)	19 (95%)		13 (65%)	7 (35%)	
Tumor stage (TNM)	Stage I, II	14	9 (64.3%)	5 (35.7%)	< 0.001	2 (14.3%)	12(85.7%)	0.012*
	Stage III	16	2 (12.5%)	14(87.5%)		6 (37.5%)	10(62.5%)	
	Stage IV	20	1 (5%)	19 (95%)		13 (65%)	7 (35%)	

EphB2, Ephrin type B receptor 2; CADM4, Cell adhesion molecule 4; * significant; ** highly significant.

Table 4: Relation between CADM4 expression in the positive gastric carcinoma cases and clinico-pathological features.

Clinico-pathological features		Total	Positive CADM4 expression		P-value
			Low expression	High expression	
Tumor subtype	**Tubular adenocarcinoma**	**23**	**8 (34.8%)**	**15 (65.2%)**	**0.811**
	Poorly cohesive carcinoma	**4**	**2 (50%)**	**2 (50%)**	
	Mucinous adenocarcinoma	**2**	**1 (50%)**	**1 (50%)**	
Tumor grade	**Low grade**	**21**	**7 (33.3%)**	**14 (66.7)**	**0.433**
	High grade	**8**	**4 (50%)**	**4 (50%)**	
Depth of tumor invasion (T)	**T1**	**6**	**1 (16.7%)**	**5 (83.3%)**	**0.027***
	T2	**3**	**0**	**3 (100%)**	
	T3	**9**	**2 (22.2%)**	**7 (77.8%)**	
	T4	**11**	**8 (72.7%)**	**3 (27.3%)**	
Lymph node metastasis (N)	**N0**	**4**	**1 (25%)**	**3 (75%)**	**0.226**
	N1	**4**	**0**	**4 (100%)**	
	N2	**15**	**6 (40%)**	**9 (60%)**	
	N3	**6**	**4 (66.7%)**	**2 (33.3)**	
Distant metastasis (M)	**M0**	**22**	**5 (22.7%)**	**17 (77.3%)**	**0.006****
	M1	**7**	**6 (85.7%)**	**1 (14.3%)**	
Tumor stage (TNM)	**Stage I, II**	**12**	**1 (8.3%)**	**11 (91.7%)**	**0.003****
	Stage III	**10**	**4 (40%)**	**6 (60%)**	
	Stage IV	**7**	**6 (85.7%)**	**1 (14.3%)**	

CADM4, Cell adhesion molecule 4; * significant; ** highly significant.

Table 5: Relation between EphB2 and CADM4 expressions in studied gastric carcinoma cases.

Gastric carcinoma (N=50)		CADM4 expression		P-value
		Negative	Positive	
EphB2 expression	**Negative (N=12)**	**3 (25%)**	**9 (75%)**	**0.201**
	Positive (N=38)	**18(47.4%)**	**20 (52.6%)**	

EphB2, Ephrin type B receptor 2; CADM4, Cell adhesion molecule 4; N, number.

Table 6: Validity of EphB2 and CADM4 expression in prediction of gastric carcinoma from non neoplastic and precursor lesions and prediction of gastric dysplasia and carcinoma from non dysplastic lesions.

EphB2 expression	**Non neoplastic / precursor lesions**		**Gastric carcinoma**	
	N	%	No	%
Positive	8	36.4	38	76
Negative	14	63.6	12	24
AUC (95%CI)	0.698 (0.558-0.882)			
Sensitivity	76			
Specificity	36.4			
EphB2 expression	**Non dysplastic lesions**		**Gastric dysplasia and carcinoma**	
	N	%	No	%
Positive	4	25	42	75
Negative	12	75	14	25
AUC (95%CI)	0.750 (0.558-0.882)			
Sensitivity	75			
Specificity	25			
CADM4 expression	**Non neoplastic / precursor lesions**		**Gastric carcinoma**	
	N	%	No	%
Positive (low)	9	40.9	11	22
Positive (high)	10	45.5	18	36
Negative	3	13.6	21	42
AUC (95%CI)	0.382 (0.558-0.882)			
Sensitivity	58			
Specificity	86.4			
CADM4 expression	**Non dysplastic lesions**		**Gastric dysplasia and carcinoma**	
	N	%	N	%
Positive (low)	5	31.3	15	26.8
Positive (high)	10	62.5	18	32.1
Negative	1	6.3	23	41.1
AUC (95%CI)	0.292 (0.558-0.882)			
Sensitivity	58.9			
Specificity	93.8			

EphB2, Ephrin type B receptor 2; CADM4, Cell adhesion molecule 4;
AUC, area under the curve N, number.

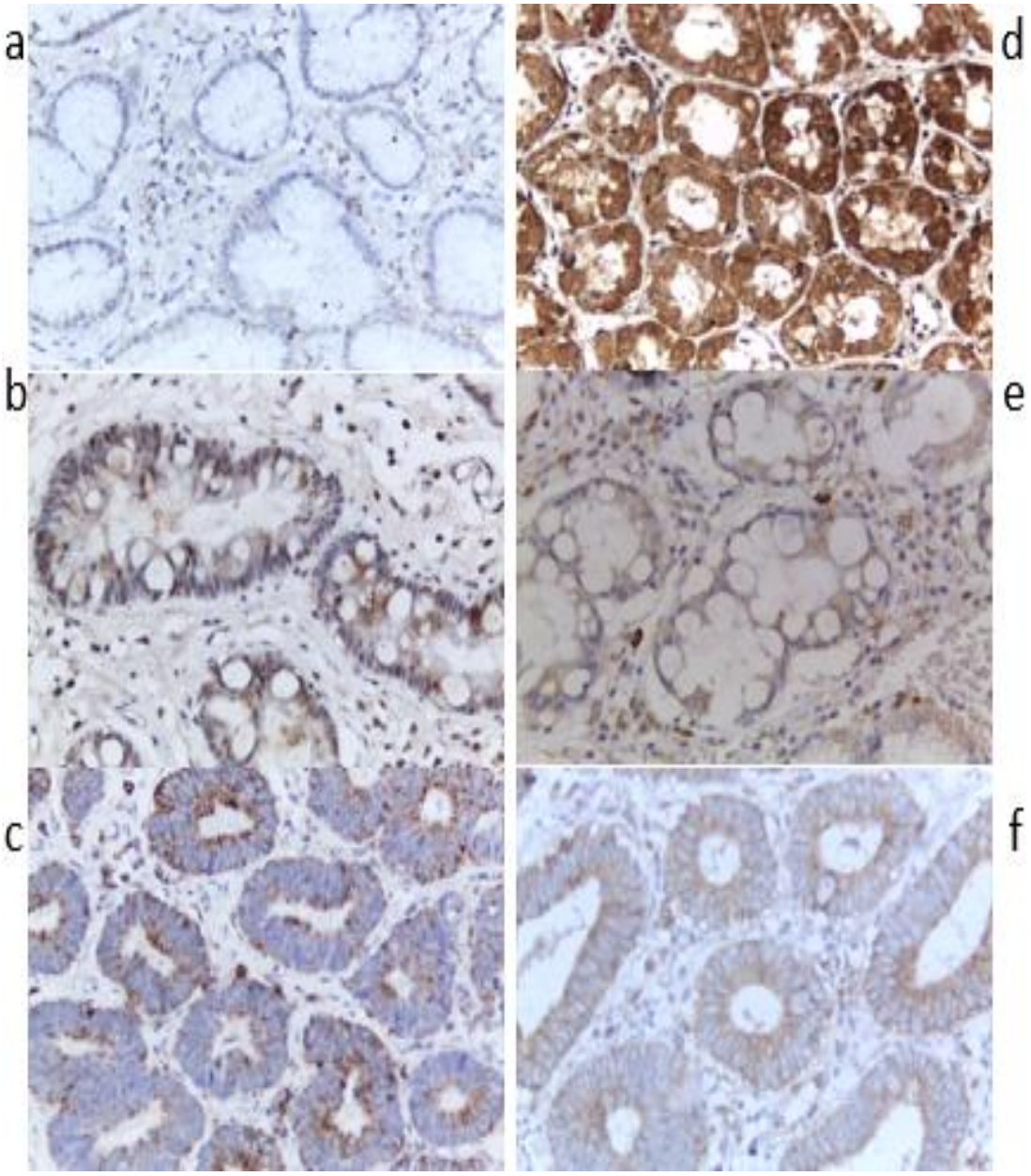

Figure (1): a- chronic gastritis showing negative EphB2 expression score 0 (ABCx400). b- Chronic gastritis with intestinal metaplasia showing positive EphB2 expression score 4 (ABCx200). c- Gastric adenoma with low grade dysplasia showing positive EphB2 expression score 3 (ABCx400). d- Chronic gastritis showing positive CADM4 expression score 12 (high expression) (ABCx400). e- Chronic gastritis with intestinal metaplasia showing positive CADM4 expression score 3 (low expression) (ABCx400). f- Gastric adenoma with low grade dysplasia showing positive CADM4 expression score 3 (low expression) (ABCx200).

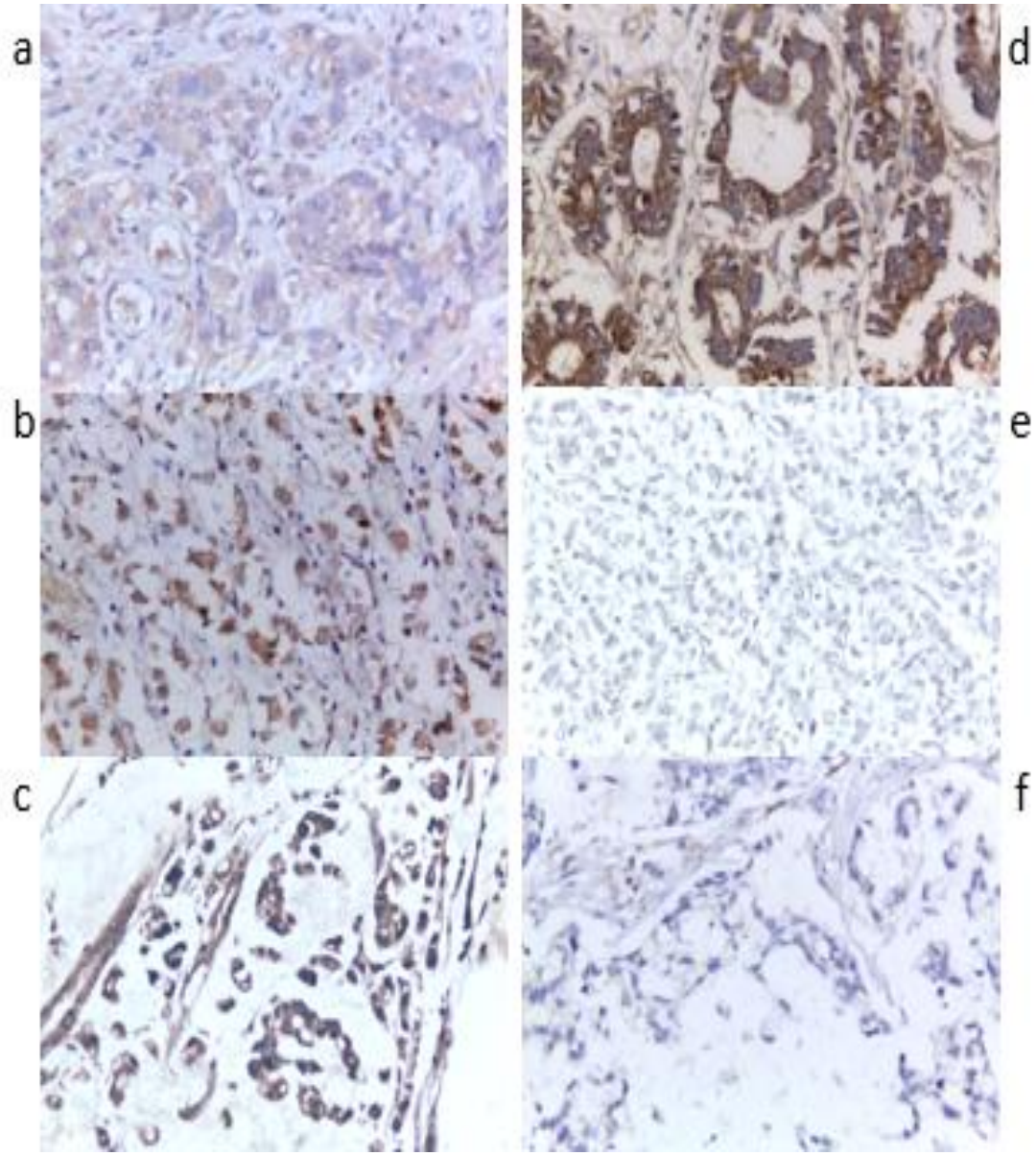

Figure (2): a- Tubular adenocarcinoma showing positive EphB2 expression score 4 (ABCx400). b- Poorly cohesive carcinoma showing positive EphB2 expression score 5 (ABCx400). c- Mucinous adenocarcinoma showing positive EphB2 expression score 6 (ABCx400). d- Low grade tubular adenocarcinoma showing positive CADM4 expression score 9 (high expression) (ABCx400). e- Poorly cohesive carcinoma showing negative CADM4 expression score 0 (ABCx400). f- Mucinous adenocarcinoma showing negative CADM4 expression score 0 (ABCx400).

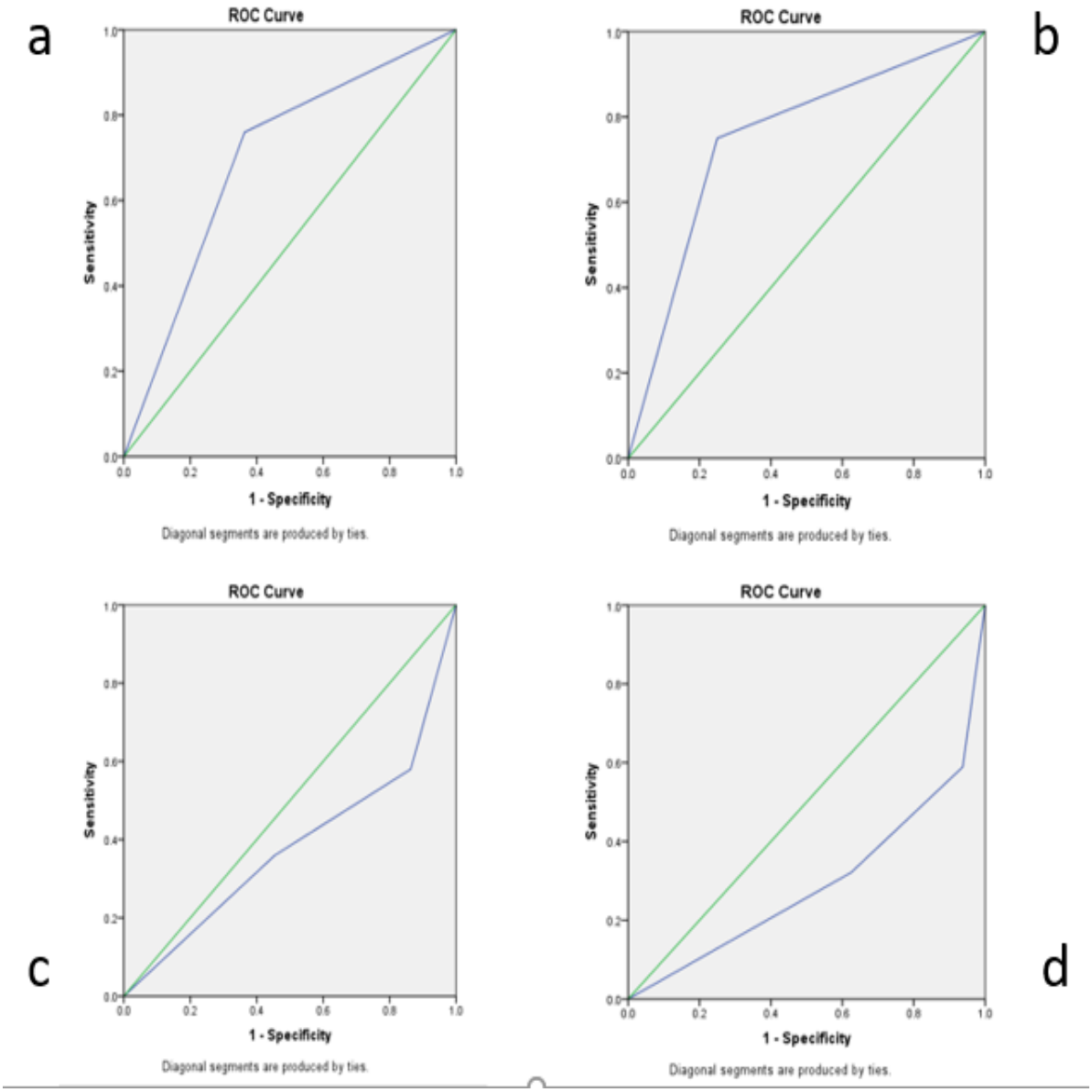

Figure (3): Graphs of ROC curve analysis to determine a- Validity of EphB2 in prediction of gastric carcinoma from non neoplastic and precursor lesions. b- Validity of EphB2 in prediction of gastric dysplasia and carcinoma from non dysplastic lesions. c- Validity of CADM4 in prediction of gastric carcinoma from non neoplastic and precursor lesions. d- Validity of CADM4 in prediction of gastric dysplasia and carcinoma from non dysplastic lesions.

Discussion:

The study groups of the current study demonstrated a highly significant statistical difference in EphB2 expression (P value = 0.002). The GC cases showed the highest percentage of positive EphB2 expression (76%), while 20% of chronic gastritis, 33.3% of chronic gastritis with intestinal metaplasia and 66.7% of gastric adenomatous lesions showed positive EphB2 expression. EphB2 expression was negative in the apparently normal gastric tissue. The study performed by Yin et al., [14] was in line with these results. They observed that the scores of EphB2 expression were significantly higher in GC than adjacent tissue and chronic gastritis

specimens. These results suggest that EphB2 upregulation may be a significant factor in the development of GC with a possible diagnostic potential.

In correspondence with the current study, Jang et al., [17] reported that EphB2 expression was upregulated in colorectal carcinoma cases compared to non-cancerous tissue. Also, Leung et al., [18] reported that the expression of EphB2 was elevated with progression from normal liver to fibrotic liver to hepatocellular carcinoma (HCC). Furthermore, Peng et al., [19] demonstrated that EphB2 expression was significantly higher in oral squamous cell carcinoma than in normal mucosa.

In the current study, EphB2 expression was significantly associated with increased depth of tumor invasion (P-value = 0.006), lymph node metastasis (P-value = 0.026), distant metastasis (P-value = 0.016) and high TNM stage of GC (P-value < 0.001). These results are in agreement with Yin et al., [14], Ebrahim et al., [20] and Peng et al., [19] who concluded a significant association between EphB2 expression and advanced stage of GC, breast carcinoma and oral squamous cell carcinoma, respectively.

These results suggest that EphB2 may have a role in tumor progression and epithelial mesenchymal transition (EMT) facilitating tumor migration and invasion. These findings support the potential value of EphB2 as a marker for invasion and metastasis in GC. This could be attributed to the impact of EphB2 on the adhesive and migration capabilities of GC cell lines. The adhesion capacity of GC cells decreased and their migration capacity increased by activation of EphB2 [14]. Also, EphB2 modulated cancer stem cell (CSC) like characteristics in certain tumors such as HCC and cervical carcinoma. EphB2 promoted self-renewal, tumorigenicity and drug resistance in HCC. EphB2 drived these features in HCC through Wnt/β-catenin signaling [18]. In cervical carcinoma, EphB2 promoted CSC characteristics through activation of EMT through R-Ras signaling [21].

In contrast, Jang et al., [17] reported that positive EphB2 expression was inversely correlated with depth of tumor invasion, lymph node metastasis, distant metastasis and advanced TNM stage of colorectal carcinoma. Similarly, Lee et al., [22] reported that low EphB2 expression was significant associated with muscle invasion and high stage of bladder carcinoma. Furthermore, Kim et al., [23] reported that positive EphB2 expression was significantly associated with lower TNM stage of GC in Korean patients. This contrast could be explained as EphB2 performs its regulatory functions in several ways depending on the specific organ, tumor type and patient race. It may act as a tumor promoter. Also, it may act as a tumor suppressor [7].

A statistically insignificant difference (P-value =0.344) was observed in the relationship between EphB2 expression and GC histopathological subtype. About 69% of tubular adenocarcinoma, 91.7% of poorly cohesive carcinoma and 83.3% of mucinous adenocarcinoma cases were positive for EphB2 expression. These results agreed with Zhao et al., [24] and Yin et al., [14] in their studies on lung adenocarcinoma and GC, respectively.

There was a statistically insignificant difference in the expression of EphB2 between the analyzed cases with respect to tumor grade (P-value = 0.201). These results are in line with the results concluded by Jang et al., [17] and Yin et al., [14] in their studies on colorectal carcinoma and GC, respectively. In contrast Kim et al., [23] reported that positive EphB2 expression was significantly higher in low grade than in high grade GC in Korean patients. This discrepancy may be attributed the geographic and genetic variability among races and their larger number of cases.

The study groups of the current study showed a highly significant statistical difference in CADM4 expression (P value

< 0.001). The GC cases showed the highest percentage of negative CADM4 expression (42%), 22% showed low expression and 36% showed high expression. All cases of chronic gastritis, 83.3% of chronic gastritis with intestinal metaplasia and 66.7% of gastric adenomatous lesions showed positive CADM4 expression. CADM4 expression was positive in the apparently normal gastric tissue. These present results are in alignment with Bang et al., [8] who reported that CADM4 expression was lost or reduced in about 57% of GC cases. These results suggest that loss of CADM4 may be an early step in carcinogenesis and highlight a possible diagnostic significance.

Regarding the relation between CADM4 expression and histopathological subtype, 71.9% tubular adenocarcinoma cases were positive for CADM4 expression, while 66.7% of poorly cohesive carcinoma and 66.7% of mucinous adenocarcinoma cases were negative with a significant statistical difference (P-value = 0.032). These results are in agreement with Bang et al., [8] who discovered that diffuse and mixed types of GC were significantly associated with absent and low CADM4 expression.

Another study performed by Kim et al., [16] observed that negative CADM4 expression in small intestinal adenocarcinoma was significantly associated with undifferentiated subtype. These results suggest that loss of CADM4 expression is more frequently associated with aggressive histopathological subtypes which may be attributed high grade of these subtypes.

In the current study, 72.4% of low-grade cases were positive for CADM4 expression, while 61.9% of high-grade cases were negative. A significant inverse statistical relation was observed between CADM4 expression in GC cases and tumor grade (P-value = 0.015). These results are in line with Kim et al., [16]. They observed that high-grade small intestinal adenocarcinoma was significantly associated with the loss of CADM4 expression. This could be attributed to altered and disrupted cell adhesion molecules expression in high grade tumors leading to disorientation of cells and tumor progression [25].

A highly significant inverse statistical relation was observed between CADM4 expression and distant metastasis (P-value = 0.007), as well as between CADM4 expression among the positive cases and distant metastasis (P-value = 0.006). Furthermore, there was a significant inverse statistical relation between CADM4 expression and TNM stage (P-value = 0.012) and even a highly significant inverse statistical relation between CADM4 expression among the positive cases and TNM stage (P-value = 0.003). These results are in agreement with Bang et al., [8] who found that high stage of GC was significantly associated with the lost and reduced expression of CADM4.

Also, the studies performed by Saito et al., [26] and Bang et al., [27] reported that low expression of CADM4 was significantly associated with advanced tumor stage of invasive breast carcinoma and gall bladder adenocarcinoma, respectively.

The mechanism of this role remains uncertain. However, it was reported that CADM4 interacts with other surface molecules in the extracellular matrix. The interaction between CADM4 and VEGF receptors is thought to maintain contact inhibition by inhibiting tyrosine phosphorylation of VEGF receptors through protein-tyrosine phosphatase, non-receptor type 13 (PTPN13). Furthermore, the disassembly of hemidesmosomes is a consequence of CADM4 inhibition in tumor cells, which in turn facilitates the invasion and metastasis of tumor cells [27].

A statistically insignificant difference was observed between CADM4 expression and depth of tumor invasion (P-value = 0.149). However, there was a significant inverse statistical relation between CADM4 expression in the positive cases and depth of tumor invasion (P-value = 0.027). High

expression was observed in approximately 83% of T1, all T2 cases, and 78% of T3 cases, while low expression was observed in 66.7% of T4 cases. These results are supported by the findings reached by Kim et al., [16], Bang et al., [8] and Bang et al., [27] who observed significant associations between low CADM4 expression and advanced tumor extent in small intestinal adenocarcinoma, GC and gall bladder adenocarcinoma cases, respectively.

The expression of CADM4 in studied cases did not exhibit a statistically significant difference in terms of lymph node metastasis (P-value = 0.196). These findings are consistent with Kim et al., [16] and Bang et al., [27] who observed no significant correlation between CADM4 expression and lymph node metastasis in small intestinal adenocarcinoma and gall bladder adenocarcinoma respectively.

In contrast, Bang et al., [8] reported that absent and low CADM4 expression was significantly associated with nodal metastasis in GC. The wider scale of these studies and different methods of investigations may be the reasons for this controversy.

The current study demonstrated a statistically insignificant relation between EphB2 and CADM4 expression in GC cases (P-value = 0.201) The ROC curve analysis revealed that EphB2 was more sensitive than specific, while CADM4 was more specific than sensitive in predicting GC from non neoplastic and precursor lesions as well as in predicting gastric dysplasia and carcinoma from non dysplastic lesions. These results indicate that EphB2 and CADM4 operate through distinct mechanisms in the development and progression of GC. No comparable published data regarding the relationship between EphB2 and CADM4 expression in GC or other carcinomas are available.

However, there was a study performed by Zhao et al., [24] concerning the relation between EphB2 and junctional adhesion molecule-A (JAM-A) expression in lung adenocarcinoma. JAM-A is also one of the immunoglobulin superfamily molecules. This study reported that elevated expression of JAM-A and EphB2 were significantly correlated with poor overall survival and could predict poor outcome and high mortality of lung adenocarcinoma.

Conclusion

The development and progression of GC may be influenced by the upregulation of EphB2. Loss of CADM4 expression could be an early step in gastric carcinogenesis and its downregulation may be linked to adverse prognostic factors. EphB2 and CADM4 may serve together as beneficial prognostic markers and therapeutic targets for GC patients. Further wider scale studies are recommended to further clarify the role of EphB2 and CADM4 in GC and to study the therapeutic potential of targeting EphB2 and CADM4 in GC.

Conflicts of interest:

The authors announce that they have no competing interest.

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