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Original Article

E-cadherin/β-catenin expression pattern, clinicopathological parameters and prognosis in gastric carcinomas

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Abstract

Objectives: This study aimed to evaluate the expression pattern of some markers (E-cadherin and β-catenin) related to cellular adhesion and their relationship with histological tumor type according to Laurén's system, clinicopathological features and patient survival. **Material and Methods:** We did immunohistochemical analysis in a retrospective series of 446 gastric carcinomas using tissue microarray method (TMA). Clinicopathological features and overall survival data of all patients were retrospectively reviewed from hospital records. For all statistical analyses, $p<0.05$ was considered significant. **Results:** The reduced/absent expression of E-cadherin occurred more frequently in diffuse than intestinal type tumors and it was correlated with worse biological behavior and poor prognosis for patients with diffuse type gastric carcinomas. The pattern of β-catenin expression was closely related to histological type and E-cadherin expression. Although patients with nuclear/absent β-catenin immunoreactivity showed worse survival index, no statistical correlation was found with overall survival. In multivariated analysis, only pTNM staging system persisted as independent prognostic marker. **Conclusion:** In the present study, alterations in E-cadherin/β–catenin complex expression showed significant correlations with clinicopathological parameters, as well as its implications for tumor progression and prognosis in gastric cancer. Our results indicate that markers expression pattern may be a useful marker of differentiation and suggest further investigations of their prognostic relevance to specific histological groups.

Key words: E-caderin. β-catenin. Gastric neoplasms.

Introduction

Gastric carcinomas are a heterogeneous group of tumors, considering epidemiology, genetics, histopathlogy and biological behavior, and are thought to result from a combination of environmental factors and the accumulation of specific genetic alterations. According to Laurén´s histological classification, they can be divided into diffuse- and intestinal- type.[1] These two types have differences in pathology, epidemiology and etiology. Regarding the differences between both groups, some investigators from our institution compared differential expression profiles in gastric adenocarcinomas using cDNA microarrays and identified genes that are differentially expressed between diffuse and intestinal type gastric carcinomas.[2]

Advances in molecular biology have reported the involvement of cell adhesion molecules in neoplastic transformation. Abnormalities in expression and function of the adhesion complex - mainly in E-cadherin and β-catenin - lead to dysfunctions of intercellular adhesion, which may be accompanied by higher mobility of tumor cells and aggressive tumor behaviors.[3,4] E-cadherin is a central component of the adherens junction and its

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association with cellular cytoskeleton proteins, α- and β-catenin, is indispensable for cell-cell adhesion and maintainance of normal tissue architecture.[5,6] Inactivation of E-cadherin is an important step in the development of most epithelial-derived tumor types[7] and its loss is associated with an infiltrating phenotype and poor prognosis.[8-11] It has been suggested that loss of E-cadherin is the fundamental deficiency in diffuse type gastric cancer, and provides an explanation for observed morphological phenotype such as cells with loss of cohesion, polarity and gland architecture.[12] Also, in familial gastric cancer, there are no mutations in cases of intestinal morphology, but E-cadherin inactivation was common in diffuse type.[13] These findings suggest there may exist different genetic pathways for intestinal- and diffuse-type gastric cancers.[14,15] Somatic alterations of E-cadherin gene have been reported in sporadic diffuse gastric cancers but not in intestinal type gastric cancers.[16-19] Germline mutations in the *CDH1* gene (E-cadherin) have been associated with familial gastric cancer.[20-24]

β-catenin is a multifunctional protein, and plays an important role in Wnt signal transduction in addition to its function as a cell adhesion system component.[4] According to previous reports, the increased free β-catenin in the cytoplasm is target for destruction in the ubiquitin-proteasome system or may translocate to the nucleus and act as a transcription factor.[25,26] Mutations in β-catenin gene have been demonstrated in intestinal.[27,28] but not in diffuse-type carcinomas, but protein abnormalities are relatively frequent and occur in both diffuse and intestinal cancers.[29-31]

The aim of this study is to evaluate the expression pattern of E-cadherin/β-catenin complex in diffuse- and intestinal type of gastric adenocarcinomas by immunohistochemistry and Tissue Microarray (TMA) methods and to investigate the relationship of their expression pattern with histological tumor type (according to Laurén´s classification) and clinicopathological variables as age, gender, tumor size, tumor differentiation, pTNM stage, depth of invasion (T), lymph node metastasis (N) and overall survival.

Matherial and Methods

Patients and Tumor Samples

A total of 446 patients admitted to Hospital do Cancer A. C. Camargo (São Paulo, Brazil) for management of adenocarcinoma of the stomach between 1988 and 1998 were selected, and clinicopathological features of these patients were reviewed retrospectively, including information on each patient's age, gender, tumor size, tumor differentiation, TNM stage, depth of invasion (T), nodal involvement (N) and histological tumor type. Tumors were classified in three main groups: intestinal-, diffuse- and mixed/unclassified- gastric adenocarcinomas, according to Laurén's classification.[1] Pathological stage was classified according to the Union International Contre le Cancer (UICC): (TNM - tumor, node, metastasis system)[32] and stages IA and IB were grouped into one category for statistical analyses. Tumor size was categorized according to the medium size of all tumors (≤6cm and >6cm). Follow up data were obtained from hospital records. Overall survival was defined as the time elapsed from primary treatment and death from gastric cancer or other causes.

Tissue Microarray Method and Immunohistochemistry

Formalin-fixed, paraffin wax-embedded tissues from 446 gastric carcinomas were retrieved from the archival tissue bank of the Department of Anatomic Pathology. A section from each specimen was stained with haematoxylin and eosin (H&E) for histological evaluation and selection of morphologically representative sites in the tumors to construct tissue microarray blocks. Our series included 401 cases from TMA blocks and other 45 cases analyzed in conventional sections. For TMA blocks construction, tissue core biopsies (diameter 0.6mm) were punched from selected regions of donor paraffin-embedded tumor blocks and precisely arrayed into a new recipient paraffin block using a precision instrument (Beecher Instruments®, Silver Spring, MD). All the cases were spotted twice in each TMA block and immunohistochemistry was carried out in two slides in different depth levels. Immunohistochemistry analysis was performed in 3μ thick sections from each sample of tissue microarray block and conventional blocks by the standard streptavidin-biotin peroxidase technique. Briefly, tissue sections were deparaffinized and rehydrated and antigen retrieval was performed by pressure cooker in citrate buffer (0.01M, pH 6.0). Endogenous peroxidase was blocked using 3% hydrogen peroxide and then washed with phosphate-buffered saline (PBS), pH 7.4. The sections were incubated with one of the primary mouse monoclonal antibodies overnight at 4°C: E-cadherin (1:750, BD Transduction, USA) and β-catenin (1:1000, BD Transduction, USA). The sections were

then incubated during 30min at room temperature with biotinylated secondary antibodies (DAKO A/S, K492, Denmark), followed by three washes in PBS and incubated with 3.3-diaminobenzidine-tetrahydrochloride solution for 5 minutes. The sections were counterstained with haematoxylin. For negative control preparations, primary antibodies were omitted from the reaction sequence.

The expression pattern of E-cadherin and β-catenin in malignant cells was compared with that of normal cells. In addition, the staining pattern of E-cadherin was classified into three groups: membranous (when cell membrane of tumor cells was stained as strongly as normal epithelial cells), reduced (when cell-membrane staining exhibited a very weak or dotted expression) and absent patterns (a complete loss of staining). For β-catenin, four staining pattern could be discerned: the membranous and absent patterns (as described for E-cadherin), cytoplasmic pattern (cytoplasmic staining with/without loss of membranous expression) and nuclear staining pattern. In the case of mixed patterns in some sections, the classification was based on the dominant pattern.

Statistical Analyses

All statistical analyses were performed using Stata, version 7.0, statistical software program. Chi-square test and Fisher's exact test were used to analyse the association between clinicopathological parameters and molecular biomarkers expression. The five-year survival rates were estimated using the Kaplan-Meier method, and the log-rank test was used to compare the curves. Cox proportional hazards model was performed to find the independent risk factors for death. For all tests, $p<0.05$ was considered significant.

Results

Clinicopathological parameters and markers expression pattern

This study group comprised a total of 446 patients, including 266 males (59.7%) and 180 females (40.3%). Mean age was 61.2 ± 12.6yr (median 63yr) with a range from 25 to 84yr. Based on anatomopathological data, tumor size was evaluated in 428 cases. Mean size of tumors was 6.3 ± 3.3cm (median 6cm).

Regarding histological type, 185 surgical samples were classified as diffuse type (41.5%) and 261 samples (58.5%) as intestinal type gastric carcinomas. The clinicopathological parameters and markers expression analysis of all tumors according to histological type are in Table 1.

The diffuse- and intestinal type groups had similar distributions as regards tumor size, stage and depth of invasion (T). For purposes of statistical analyses, as only fours cases (0.2%) in our casuistic were classified as T4, they were grouped into the same category of T3. The diffuse type group presented a higher percentage of patients with ≤63yr ($p<0.01$), and although male patients were predominant in both groups, there was a higher frequency of female in the diffuse type group when compared to the intestinal group ($p=0.01$). As expect, considering Laurén´s classification, the group of diffuse type group was dominated by poorly differentiated tumors (92.2%, $p<0.01$). Regarding the presence of lymph node metastasis, we observed a higher frequency of N0 and N1 in intestinal type group when compared to diffuse group ($p<0.01$).

The comparison between markers expression pattern are in Figures 1-2. The diffuse type group presented higher frequency of cases with E-cadherin absent expression pattern than intestinal type group (51.7% vs 17.2%, respectively. $p<0.01$). We observed only few cases with preserved membranous β-catenin expression in both histologic groups: 5.2% of diffuse type and 8.3% of intestinal type gastric carcinomas. Moreover, β-catenin nuclear expression pattern was higher in diffuse (41.6%) in relation to intestinal type group (26.7%) ($p<0.01$).

The analysis of clinicopathological parameters according to E-cadherin expression pattern is in Table 2. Tumors with E-cadherin absent expression, were mainly poorly differentiated ($p<0.01$) and with a high percentage of lymph node involvement ($p=0.03$). According to histological classification, the absent expression of E-cadherin was more frequent in diffuse than in intestinal type carcinomas ($p<0.01$). There was a significant association between E-cadherin and β-catenin absent expression pattern and also nuclear expression of β-catenin occurred more frequently in tumors with absence of E-cadherin ($p<0.01$).

Concerning the same analysis for β-catenin expression pattern (Table 3), there was a significant association between β-catenin and E-cadherin absent expression. Also, β-catenin absent expression was associated with diffuse type tumor ($p<0.01$).

Overall Survival Analysis (OS)

Follow up ranged between one day and 171.6

Table 1 – Clinicopathological parameters and markers expression according to histological tumor type in gastric carcinomas

Variables	n	Categories	Histological Type		p-value
			Diffuse N (%)	Intestinal N (%)	
Age	446	≤ 63 yrs	114 (61.6)	111 (42.5)	<0.01
		> 63 yrs	71 (38.4)	150 (57.5)	
Gender	446	Female	88 (47.6)	92 (35.3)	0.01
		Male	97 (52.4)	169 (64.7)	
Tumor size	428	≤ 6cm	98 (55.4)	139 (55.4)	0.99
		> 6cm	79 (44.6)	112 (44.6)	
Differentiation	401	Well	0 (0.0)	33 (14.1)	<0.01
		Moderate	12 (7.2)	158 (67.2)	
		Poor	153 (92.2)	44 (18.7)	
Stage	368	IA / IB	18 (11.8)	31 (14.4)	0.41
		II	27 (17.6)	39 (18.1)	
		IIIA	38 (24.8)	63 (29.3)	
		IIIB	29 (19.0)	26 (12.1)	
		IV	41 (26.8)	56 (26.1)	
T	446	T1	18 (9.7)	40 (15.3)	0.19
		T2	38 (20.6)	45 (17.2)	
		T3 + T4	129 (69.7)	176 (67.5)	
N	441	N0	53 (28.8)	83 (32.3)	<0.01
		N1	53 (28.8)	121 (47.1)	
		N2	54 (29.3)	42 (16.3)	
		N3	24 (13.1)	11 (4.3)	
E-cadherin	424	absent	90 (51.7)	43 (17.2)	<0.01
		reduced	64 (36.8)	107 (42.8)	
		membranous	20 (11.5)	100 (40.0)	
β-catenin	424	absent	38 (22.0)	25 (10.0)	<0.01
		membranous	9 (5.2)	21 (8.3)	

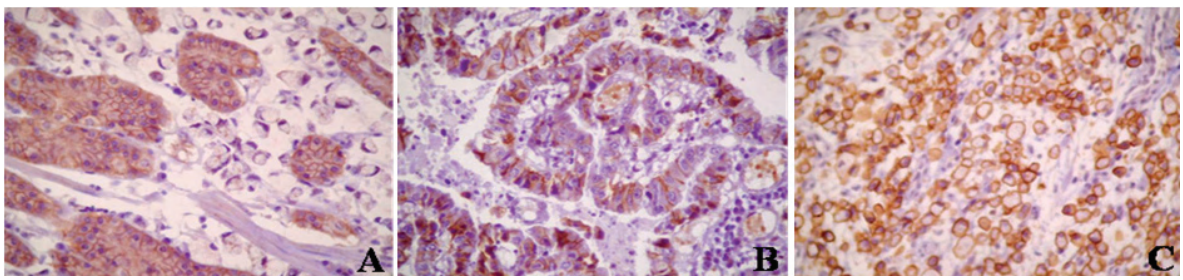

Figure 1 - Representative immunohistochemistry staining for E-cadherin expression pattern in gastric carcinomas. A. E-cadherin absent expression pattern in diffuse type gastric carcinoma – dispersed neoplasic cells displaying loss of membranous expression of E-cadherin when compared to preserved cell membrane staining in the residual gastric glands. B. E-cadherin reduced expression pattern, membranous staining can be seen in a few neoplasic cells. C. Preserved membranous expression pattern of E-cadherin

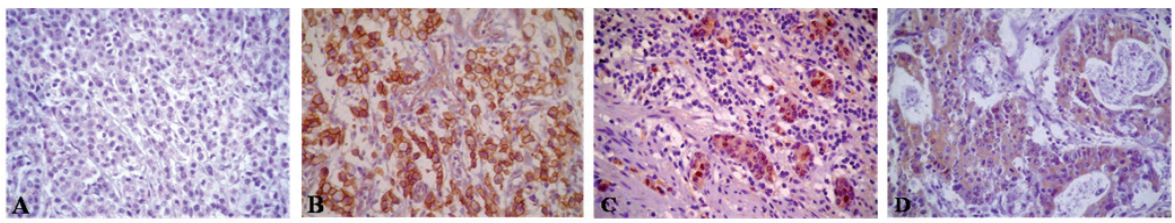

Figure 2 - Representative immunohistochemistry staining for β-catenin expression pattern in gastric carcinomas. A. β-catenin absent expression pattern in gastrica carcinoma. B. Neoplasic cells displaying β-catenin preserved membranous expression pattern. C. Nuclear staining pattern with cytoplasmic distribution of β-catenin in neoplasic cells. D. showing cytoplasmic staining pattern of β-catenin without nuclear staining

Table 2 – Clinicopathological variables and β-catenin expression according to E-cadherin expression pattern in gastric carcinomas

Variables	n	Categories	E-CADHERIN Absent N (%)	Reduced N (%)	Membranous N (%)	p-value
Age	424	≤ 63 yr	69 (51.9)	89 (52.0)	55 (45.8)	0.52
		> 63 yr	64 (48.1)	82 (48.0)	65 (57.2)	
Tumor size	407	≤6cm	70 (56.9)	87 (52.1)	65 (55.6)	0.69
		> 6cm	53 (43.1)	80 (47.9	52 (44.4)	
Tumor Differentiation	380	Well	5 (4.1)	15 (9.3)	13 (13.3)	<0.01
		Moderate	35 (29.2)	69 (42.6)	59 (60.2)	
		Poor	80 (66.7)	78 (48.1)	26 (26.5)	
Stage	348	IA / IB	14 (12.7)	19 (12.7)	11 (12.5)	0.54
		II	16 (14.6)	30 (20.0)	18 (20.4)	
		IIIA	40 (36.4)	34 (22.7)	22 (25.0)	
		IIIB	14 (12.7)	23 (15.3)	13 (14.8)	
		IV	26 (23.6)	44 (29.3)	24 (27.3)	
T	424	T1	14 (10.5)	17 (10.0)	21 (17.5)	0.29
		T2	27 (20.3)	30 (17.5)	23 (19.2)	
		T3 + T4	92 (69.2)	124 (72.5)	76 (63.3)	
N	419	Negative	34 (25.6)	50 (29.2)	47 (40.9)	0.03
		Positive	99 (74.4)	121 (70.8)	68 (59.1)	
Histological Type	424	Diffuse	90 (67.7)	64 (37.4)	20 (16.7	<0.01
		Intestinal	43 (32.3)	107 (62.6)	100 (83.3)	
β-catenin	421	Absent	43 (32.8)	14 (8.2)	4 (3.3)	<0.01
		Membranous	6 (4.6)	16 (9.4)	8 (6.7)	
		Nuclear	55 (42.0)	58 (34.1)	26 (21.7)	
		Cytoplasmic	27 (20.6)	82 (48.3)	82 (68.3)	

months, with a median follow up of 24.8 months. The overall median survival rate for all patients was 38.1% in five years. Univariate analysis of survival for all patients, regardless of histologic type, is in Table 4. Survival of patients decreased as depth of invasion (pT) and lymph node involvement (pN) increased (both $p<0.01$). Although there was no association between overall patient survival and histologic type, the analysis restricted to diffuse type gastric carcinomas (Table 5) identified a significant association between patient age ($p=0.02$), depth of invasion, lymph node metastasis (both $p<0.01$) and E-cadherin expression pattern. Specifically, E-cadherin membranous expression pattern was associated with better survival ($p=0.01$). In contrast, there was no significant association between survival and β-catenin expression pattern. In the analysis restricted to intestinal type group (Table 6), only pT and pN showed association with patient survival (both $p<0.01$). The overall survival curves according to histological type, pT and pN, and also according to E-cadherin and β-catenin expression pattern is in Figures 3 to 5. When clinicopathological parameters and markers expression were analyzed by the Cox regression model, only pTNM staging system persisted as independent prognostic markers (Table 7), even when adjusted by histological type (Table 8 and 9).

Discussion

In the current study, the comparison between histological groups showed that diffuse and intestinal type gastric carcinomas are distinct in many aspects regarding the investigated variables. Considering the characteristics according to histological tumor type, diffuse-type group contained significantly higher percentages of ≤63yr ($p<0.01$) and female patients ($p=0.01$) when compared to intestinal type group. A significant association between diffuse type tumor, poor differentiation and lymph node metastasis ($p<0.01$) was expected considering the concept of diffuse type gastric carcinomas. According to the chosen histological classification,[1] diffuse type is characterized by non cohesive neoplastic cells, mostly do

Table 3 - Clinicopathological variables and E-cadherin expression according to β-catenin expression pattern in gastric carcinomas

Variables	N	Categories	Absent	β–CATENIN Membranous	Nuclear	Cytoplasmic	p-value
Age	424	≤ 63	37 (58.7)	15 (50.0)	70 (50.4)	91 (47.4)	0.49
		> 63 yr	26 (41.3)	15 (50.0)	69 (49.6)	101 (52.6)	
Tumor Size	407	≤ 6cm	33 (56.9)	17 (58.6)	68 (50.4)	103 (55.7)	0.72
		> 6cm	25 (43.1)	12 (41.4)	67 (49.6)	82 (44.3)	
Tumor Differentiation	380	Well	1 (1.7)	2 (8.7)	12 (9.3)	19 (11.2)	NA*
		Moderate	24 (40.7)	14 (60.9)	43 (33.3)	81 (48.0)	
		Poor	34 (57.6)	7 (30.4)	74 (57.4)	69 (40.8)	
Stage	348	IA / IB	9 (17.3)	0 (0.0)	13 (10.9)	22 (14.2)	NA*
		II	6 (11.5)	4 (18.2)	18 (15.1)	34 (21.9)	
		IIIA	24 (46.2)	7 (31.8)	29 (24.4)	38 (24.5)	
		IIIB	5 (9.6)	3 (13.6)	22 (18.5)	20 (12.9)	
		IV	8 (15.4)	8 (36.4)	37 (31.1)	41 (26.5)	
T	424	T1	8 (12.7)	4 (13.3)	12 (8.6)	28 (14.6)	0.83
		T2	12 (19.1)	5 (16.7)	28 (20.2)	35 (18.2)	
		T3 + T4	43 (68.2)	21 (70.0)	99 (71.2)	129 (67.2)	
N	419	Negative	15 (23.8)	7 (23.3)	38 (27.5)	70 (37.2)	0.09
		Positive	48 (76.2)	23 (76.7)	100 (72.5)	118 (62.8)	
Histological Type	424	Diffuse	38 (60.3)	9 (30.0)	72 (51.8)	54 (28.1)	<0.01
		Intestinal	25 (39.7)	21 (70.0)	67 (48.2)	138 (71.9)	
E-cadherin	421	Absent	43 (70.5)	6 (20.0)	55 (39.6)	27 (14.2)	<0.01
		Reduced	14 (22.9)	16 (53.3)	58 (41.7)	82 (42.9)	

Table 4 – Overall survival rates of patients with gastric carcinoma according to clinicopathological variables and markers expression pattern

Variables	N	Categories	Overall Survival (%) 5 years (%)	10 years (%)	p-value
Age	225	≤ 63 yr	40.20	25.16	0.24
	221	> 63 yr	36.00	21.78	
Histological Type	185	Diffuse	38.37	23.58	0.60
	261	Intestinal	37.94	23.58	
T	58	T1	80.7	62.8	<0.01
	83	T2	60.5	43.4	
	305	T3 + T4	24.0	10.8	
N	136	N0	70.1	48.1	<0.01
	174	N1	33.0	21.8	
	96	N2	10.4	2.3	
	35	N3	17.8	0.0	
E-cadherin	133	Absent	36.5	21.8	0.38
	171	Reduced	37.0	20.5	
	120	Membranous	38.2	30.5	
β-catenin	63	Absent	42.8	15.1	0.41
	30	Menbranous	40.3	34.9	
	139	Nuclear	33.1	18.6	

not form glands and show scattered cell growth with a lack of cell-to-cell adhesion. This morphology facilitates individual neoplastic cells infiltrating the stomach wall and also leads to lymph node involvement. In our cases,

Table 5 - Overall survival rates of patients with DIFFUSE type gastric carcinoma according to clinicopathological variables and markers expression pattern

Variables	N	Categories	Overall Survival - Diffuse Type 5 years (%)	10 years (%)	p-value
Age	114	≤ 63 yr	44.1	26.5	0.02
	71	> 63 yr	29.2	18.9	
T	18	T1	88.2	64.9	<0.01
	38	T2	66.9	42.8	
	129	T3 + T4	23.2	12.3	
N	53	N0	73.0	55.9	<0.01
	53	N1	33.7	21.1	
	54	N2	15.1	5.0	
	24	N3	13.3	0.0	
E-cadherin	90	Absent	33.8	20.7	0.01
	64	Reduced	35.0	21.3	
	20	Membranous	68.7	52.0	
β-catenin	38	Absent	42.1	18.7	0.19
	9	Preserved	60.0	60.0	
	72	Nuclear	29.4	17.0	
	54	Cytoplasmic	44.4	32.0	

Table 6 - Overall survival rates of patients with INTESTINAL type gastric carcinoma according to clinicopathological variables and markers expression pattern

Variable	N	Categories	Overall Survival In Intestinal Type 5 years (%)	10 years (%)	p-value
Age	111	< or = 63 yr	36.2	24.1	0.87
	150	> 63 yr	39.1	23.3	
T	40	T1	77.5	62.5	<0.01
	45	T2	55.4	44.5	
	176	T3 + T4	24.4	9.8	
N	83	N0	68.2	43.7	<0.01
	121	N1	32.6	21.7	
	42	N2	4.6	0.0	
	11	N3	9.1	0.0	
E-cadherin	43	Absent	42.0	25.6	0.85
	107	Reduced	38.4	20.2	
	100	Membranous	32.3	26.7	
β-catenin	25	Absent	44.8	12.3	0.89
	21	Membranous	32.2	23.0	
	67	Nuclear	37.2	20.7	
	138	Cytoplasmic	36.3	27.6	

we observed an association between histological type and lymph node involvement ($p<0.01$).

It is well known that E-cadherin/β-catenin adhesion system plays a crucial role in epithelial cell-cell adhesion and the maintenance of tissue architecture. Loss of intercellular adhesion is one of the early and critical steps in metastatic cascade.[33] Previous studies have shown that abnormal expression of cadherin-catenin complex tends to occur more frequently in diffuse-type than intestinal-type carcinoma.[31,34,35]

E-cadherin pattern expression showed in our study a significant association with some clinicopathological parameters in gastric carcinomas. Abnormal expression of E-cadherin (absent/reduced) was found in a high percentage (71.7%) of all evaluated tumors. Immunohistochemical studies have shown

Table 7 – Independent risk factors for death in patients with gastric carcinoma identified in the Cox proportional hazards model

Variable	Category	HR (95% CI)	p-value
T	T1	1.0	(ref.)
	T2	1.34 (0.8 – 2.3)	0.31
	T3 + T4	3.00 (1.8 – 4.9)	<0.01
N	N0	1.0	(ref.)
	N1	1.98 (1.4 – 2.7)	<0.01
	N2	3.80 (2.6 – 5.4)	<0.01
	N3	3.60 (2.3 – 5.6)	<0.01

Table 8 - Independent risk factors for death in patients with DIFFUSE TYPE gastric carcinoma identified in the Cox proportional hazards model

Variable	Category	HR (95% CI)	p-value
T	T1	1.0	(ref.)
	T2	1.00 (0.4 – 2.5)	0.96
	T3 + T4	2.95 (1.2 – 6.9)	0.01
N	N0	1.0	(ref.)
	N1	3.65 (2.0 – 6.5)	<0.01
	N2	5.61 (3.1 – 10.0)	<0.01
	N3	6.00 (3.2 – 11.4)	<0.01

* HR ajusted by gender and age (≤ 63yr; >63 yr).

Table 9 - Independent risk factors for death in patients with INTESTINAL TYPE gastric carcinoma identified in the Cox proportional hazards model

Variable	Category	HR (95% CI)	p-value
T	T1	1.0	(ref.)
	T2	1.78 (0.9 – 3.6)	0.10
	T3 + T4	3.33 (1.8 – 6.3)	<0.01
N	N0	1.0	(ref.)
	N1	1.58 (1.0 – 2.4)	0.03
	N2	3.68 (2.2 – 6.1)	<0.01
	N3	2.50 (1.2 – 5.1)	0.01

abnormal expression of the complex in 30-75 per cent of gastric carcinomas.[31,36] The significant association with histologic type agrees with previous studies[31,34,37] and shows that loss of E-cadherin occurred more frequently in diffuse (51.7%) than intestinal type (17.2%) (p<0.01).

Considering these findings, we analyzed the clinicopathological variables according to each expression pattern of both markers (E-cadherin/β-catenin). We observed that E-cadherin absent expression pattern was

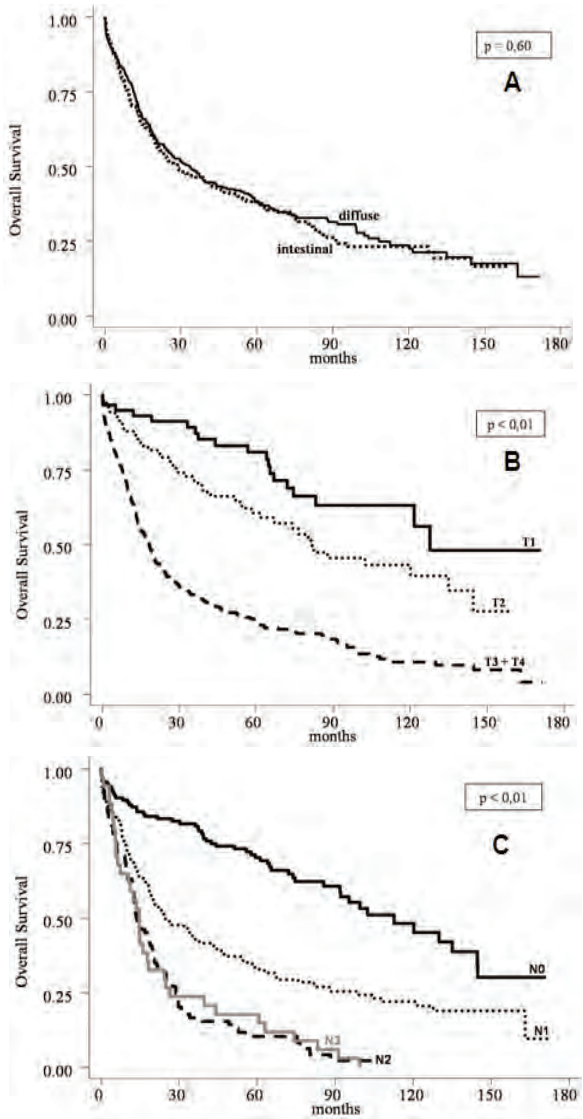

Figure 3 - Overall survival curves of 446 patients with gastric carcinomas according to A. histologic type, B. depth of invasion (T) and C. lymph node metastasis

significantly associated with poorly differentiated tumor and diffuse histological type as well (both p<0.01) as lymph node involvement (pN) (p=0.03) in contrast to some studies that reported no association with pN.[37,38]

Cadherin-mediated cell adhesion system has been shown to act as an "invasion suppressor system" in cancer cells.[39,40] and structural abnormalities of adhesion system components may lead to disruption of intercellular adhesion complex. The interactions between E-cadherin cytoplasmic domain and β-catenin represent a prerequisite not only for cell-cell adhesion, but also for inhibition of cell motility and invasion.[41]

Our results showed an association between

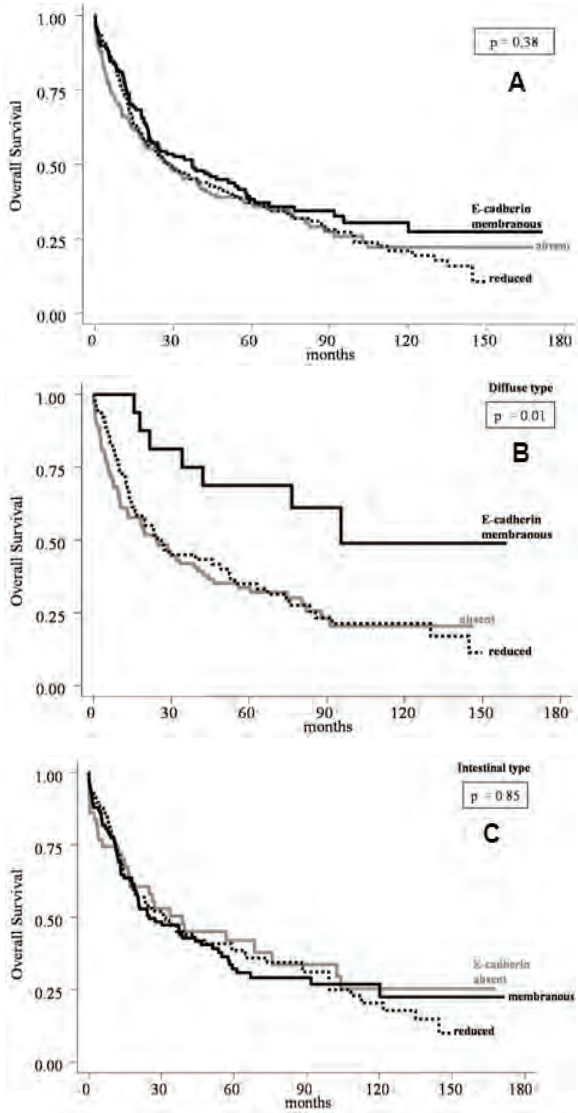

Figure 4 - Overall survival curves of 446 patients with gastric carcinomas according to E-cadherin expression pattern A. for all patients with gastric carcinoma, B. only for diffuse type and C. only for intestinal type gastric carcinomas

abnormal expression of E-cadherin and β-catenin: tumors with a lack of E-cadherin membranous staining presented a significantly higher frequency of absent (32.8%) and nuclear (42.0%) β-catenin expression pattern. β-catenin nuclear expression is very important considering the activation of a probable activaction of Wnt pathway. Detection of β-catenin mutations have been reported in 26% of gastric carcinomas displaying nuclear expression of β-catenin and no mutations were detected in tumors negative for β-catenin nuclear staining.[42] In our cases, abnormal β-catenin expression pattern (absent, nuclear) showed association only with diffuse histological type and absence of E-cadherin expression (both $p<0.01$). This findings agree with a study that found the same association with histological type[43] but contrasts with other,[44] although this same study also did not find association between β-catenin expression and tumor progression and prognosis. It was also reported significant associations between cytoplasmic and/or nuclear β-catenin expression and lymph node metastasis and lymphatic vessels invasion.[45]

In some diffuse-type gastric carcinomas, loss of cell-cell contact between neoplastic cells can be observed even when E-cadherin expression is preserved in tumor cells membranes. A similar situation has been reported in other studies in gastric cancer[29,34,46] and strongly suggests that the presence of E-cadherin as revealed by immunohistochemistry might not indicate that E-cdherin is necessarily functional. However, it is difficult to reach conclusions about the specific molecular events which are occuring from immunohistochemical studies alone. It is known that abnormal protein expression of cadherin/catenin complex components may occur for a number of reasons: gene mutations, hypermethylation of promoter region of genes, abnormal transcription and tyrosine phosphorylation of β-catenin.[9,19,47] Furthermore, E-cadherin and β-catenin is only part of a complex cell adhesion system in which the cytoplasmic domain of E-cadherin interacts with cytoskeleton through catenins.[31,48,49]

Survival analysis showed that patients with preserved E-cadherin and β-catenin expression had better overall survival rates but it was not statistically significant. A previous study reported that loss of membranous β-catenin expression, but not gain of intracellular expression, was significantly associated with poor survival.[36] Some authors found significant association between E-cadherin expression and prognosis[34,44,50] in contrast to others that did not find the same results.[31,36] Of interest, the survival analyses restricted to diffuse- and intestinal type showed a significant association between membranous E-cadherin expression pattern and better prognosis ($p=0.01$) only for diffuse type group, while precisely pT and pN showed significant association with prognosis for intestinal type. This result supports the hypothesis that distinct molecular alterations leading to the two histological types of gastric carcinomas may be different enough from each other to be considered two separate entities. In multivariated analysis, only pTNM staging system persisted as an independent prognostic marker.

In the present study, we observed a high frequency of E-cadherin and β-catenin abnormal expression in gastric cancer and a significant correlation between abnormal E-cadherin expression and clinicopathological

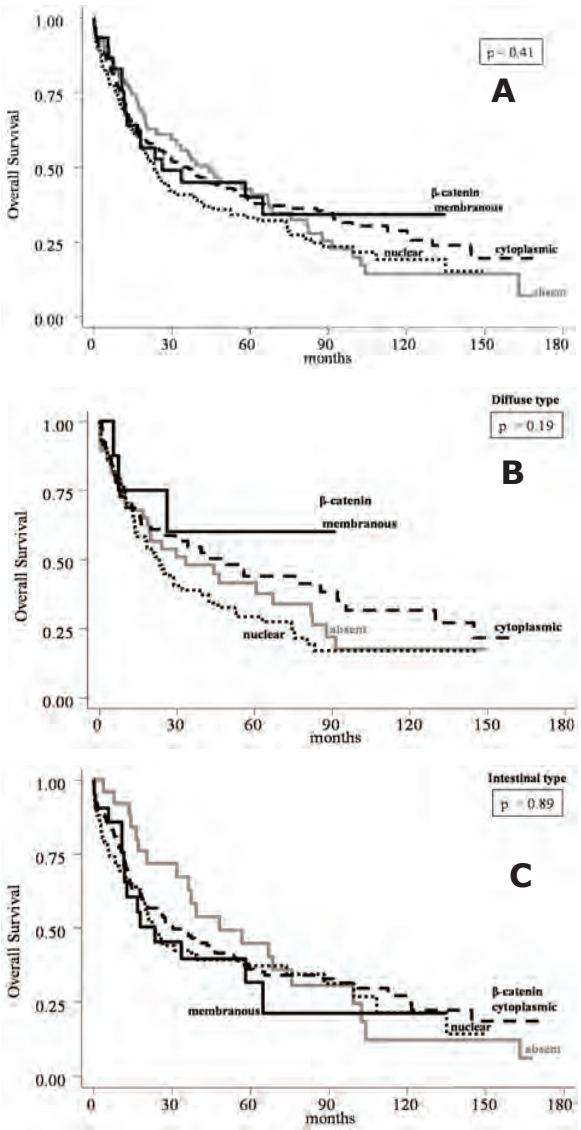

Figure 5 - Overall survival curves of 446 patients with gastric carcinomas according to β-catenin expression pattern A. for all patients with gastric carcinoma, B. only for diffuse type and C. only for intestinal type gastric carcinomas

parameters, as well as its implications for tumor progression and prognosis in diffuse type gastric cancer. We also observed that abnormal cytoplasmic/nuclear expression of β-catenin occurs in a subset of gastric carcinomas, although molecular mechanisms leading to activation of Wnt/β-catenin signaling pathway still have to be elucidated. Our results indicate that E-cadherin/β-catenin pattern expression is a useful marker for gastric adenocarcinomas and strongly suggest further investigations to access their prognostic relevance to specific histological groups.

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