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Prognostic significance of E-cadherin expression in hepatocellular carcinoma: correlations with clinical features

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Abstract

Background: Hepatocellular carcinoma (HCC) represents a major public health issue, being associated with high morbidity and mortality rates. Previous studies have demonstrated that reduction and/or absence of E-cadherin expression is correlated with a potential for invasion and low survival rate in patients with HCC. *Patients, Materials and Methods*: We assessed the immunohistochemical expression of E-cadherin in 32 HCCs and peritumoral hepatic tissues using monoclonal anti-E-cadherin antibody (clone EP700Y), at 1:50 dilution, followed by incubation with Labeled Streptavidin–Biotin 2 (LSAB2) for 20 minutes, visualization of the reaction with 3,3'-Diaminobenzidine (DAB) and counterstaining with Mayer's Hematoxylin. *Results*: The results we obtained show: an aberrant E-cadherin expression more frequent in dysplastic nodules (p=0.285) and in 81.25% of HCC cases, as compared to normal hepatic tissue (p<0.001); the absence of a statistically significant relationship between E-cadherin expression and patients' gender (p=0.854), tumor localization (p=0.429), associated viral infection [hepatitis B virus (HBV) or hepatitis C virus (HCV)] (p=0.513) or tumor size (p=0.788); the rate of positive E-cadherin expression was significantly higher in tumors with capsular infiltration (75%) (p=0.017) and does not appear to be influenced by vascular invasion (62.5%) (p=0.411), the presence of satellite nodules (p=0.285) or the serum level of alpha-fetoprotein (α-FP) (p=0.787). *Conclusions*: Reduced E-cadherin expression indicates a poor prognosis for patients with HCC and can be considered a potential predictive marker for the prognosis of these patients.

Keywords: liver, hepatocellular carcinoma, E-cadherin, immunohistochemistry.

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors and a major cause of cancer mortality in the world. Epidemiological studies have shown that over 70% of HCC cases are linked to chronic viral infections with hepatitis B virus (HBV) or hepatitis C virus (HCV). Other risk factors are alcohol consumption, correlated with development of cirrhosis, ingestion of B1 aflatoxin, in certain tropical areas, and inherited metabolic diseases, such as tyrosinemia, hemochromatosis and α_1-antitrypsin deficit. The mechanisms that lead to the development of HCC were analyzed in genomic studies, showing the presence of multiple chromosomal anomalies during tumor development and progression [1–5] and led to the identification of several risk factors for malignant hepatic tumors.

Despite considerable progress in the development of new therapies, mortality and morbidity caused by HCC remain high and the prognosis of these patients is still uncertain [6], new biomarkers being needed for anticipating the development of this tumor [7–10].

The expression of adhesion molecules, such as cadherin, catenin, selectin, integrin modifies dynamically inside the tumor and is associated with tumor invasion and metastasis, potentially representing new prognostic predictive markers in patients with HCC [11–14].

E-cadherin is an important member of the family of cellular adhesion molecules expressed by epithelial cells [15]; it is a transmembrane glycoprotein with a molecular weight of 120 kDa, which mediates the calcium-dependent intercellular adhesion. E-cadherin is anchored to the cytoskeleton through catenins and acts as a suppressor of tumor invasion and metastasis [16]. E-cadherin is expressed by most epithelial tissues and has an important role in the maintenance of cell polarity, epithelial stratification and cellular differentiation.

Previous studies have shown low protein expression of E-cadherin in a variety of tumors [17] characterized by reduced adhesion of epithelial cells and an increased motility and invasiveness of tumor cells [18–23], showing that a reduction and/or absence of E-cadherin in carcinomas is positively correlated with a potential for invasion and

metastasis of these tumors, with invasion and recurrence of HCC and a poor prognosis in patients with HCC treated by surgical resection [24]. Few studies are known to have analyzed the genetic and epigenetic changes of E-cadherin in hepatic dysplastic nodules (DN) [25].

Aim

We analyzed the prognostic significance of reduced E-cadherin expression in 32 HCCs and the surrounding normal hepatic tissue. E-cadherin expression was correlated with various clinical and pathological parameters.

Patients, Materials and Methods

Patients and tumor tissues

This paper is a retrospective study of 32 HCC cases, diagnosed in the Department of Pathology (DP) and Surgical Clinics of the Emergency County Hospital of Timişoara, Romania. Cases were selected after consulting the DP databases and patient records. We analyzed the main demographic variables (gender, age) and tumor characteristics (location, size, histological type, staging) of the patient.

Primary processing of the surgically resected specimens was done after specimen sectioning, 10% neutral buffered formalin fixation for 24–48 hours, paraffin inclusion according to the standard technique (washing, dehydration, clearing, inclusion), followed by section cutting at 4–5 μm (multiple seriated sections were made for each case).

The routine morphological examination was made on Hematoxylin–Eosin (HE)-stained sections. Microscopic examination of sections stained with HE allowed determination of the following parameters: microscopic diagnosis of the primary tumor, microscopic tumor typing [according to the *World Health Organization* (WHO) Classification] [26], grading of the tumor (G), local tumor extension (pT), status of regional lymph nodes (pN), lymphatic-vascular (L–V) and peri-/intraneural invasion, surgical resection margins (R).

Histological grading was established according to Edmondson's & Steiner's criteria (1954) [27]. HCC staging followed *American Joint Committee on Cancer* (AJCC) Criteria for cancer staging [28]. DN were histologically diagnosed according to *International Working Party* (1995) [29] criteria.

Immunohistochemistry

We investigated the immunohistochemical (IHC) expression of E-cadherin in HCC and the surrounding hepatic tissue (normal, with chronic hepatitis lesions or cirrhosis) and we assessed the correlation with clinical and morphological factors, as well as patient outcome. For the IHC study, we selected a representative block from each case; additional sections were made (4 μm thick) that were then either mounted on SuperFrost® Ultra Plus slides or silanized in order to avoid their detachment during the pretreatment process.

For E-cadherin IHC staining, we used the Labeled Streptavidin–Biotin (LSAB)+ technique. For the blocking of endogenous peroxidase, sections were treated with 3% hydrogen peroxide (H_2O_2) in methanol, for 10 minutes; then, they were immersed in 0.05 M citrate buffer (pH 6) and heated in a microwave oven for 10 minutes, at 100°C, in order to enhance antigen retrieval.

After blocking the endogenous peroxidase, sections were incubated for 30 minutes with the primary antibody – a 1:50 dilution of rabbit monoclonal anti-E-cadherin (clone EP700Y) antibody –, followed by incubation with LSAB2 for 20 minutes, visualization of the reaction with 3,3'-Diaminobenzidine (DAB) and a mild counterstaining of the nuclei using Mayer's Hematoxylin.

Evaluating the IHC staining

We observed a pattern of membranous and/or cytoplasmic E-cadherin staining.

For the evaluation of E-cadherin expression, we used a scoring system that assessed E-cadherin reactivity based on the extent and intensity of immunostaining. E-cadherin IHC expression was evaluated using o score representing the sum of positive cell percentage and staining intensity.

According to the positive cell percentage, the reactivity score was classified as follows: 0 – between 0–5% positive cells, +1 – between 5–25% positive cells, +2 – between 26–50% positive cells, +3 – between 51–75% positive cells, and +4 – between 76–100% positive cells.

The intensity score has also been divided into four groups: 0 – negative IHC reaction, +1 – mild IHC reaction, +2 – moderate IHC reaction and +3 – intense IHC reaction.

The sum of these two parameters ranged from 0 to 7.

According to the criteria proposed by Gamallo *et al.* (1993) [30], we considered tumors with a score of 6 and 7 as being E-cadherin immunoreactive (with the preservation of E-cadherin expression); E-cadherin expression was considered "absent or lost" when the total score was 0 and mildly positive (+) on a scale of 1 to 5.

We considered as normal a pattern with uniform membranous staining, whereas the uniform negative pattern and the heterogeneous pattern (cytoplasmic and membranous) were considered as an aberrant E-cadherin expression.

The expression of non-tumor hepatic tissue was used as internal control. For each case, we compared E-cadherin IHC expression in malignant cells with the intensity of the reaction in normal hepatocytes (homogeneous and intense membranous expression).

Statistical analysis

χ^2 (*chi*-square) test was used to analyze the statistical relationships between variables and results were considered statistically significant when $p<0.05$.

Ethics surrounding the scientific research

All procedures undergone in this study [histopathological (HP) analysis and microscopic images from the slides] had patient approval and a written informed consent was signed for the use of biological material in research studies (Annex No. 4 for the Law 112/2013). For the study of cases and access to the database, we had consent from the heads of DP and the Clinics involved in this study.

Results

Patient selection and assessment of the analyzed clinical and pathological parameters

We evaluated E-cadherin expression in 32 cases (with clinical and HP diagnosis of HCC); 12 men and 20 women underwent curative surgical resection for HCC, with complete tumor resection (defined by the absence of microscopic tumor cells in resection margins).

Patients' age was between 21 and 69 years, with a mean age of 56.38 years. The ratio of women to men was 1.66. In this study, we did not include patients with preoperative oncology treatments, such as chemotherapy or radiotherapy.

The tumor-free hepatic tissue surrounding the tumor was cirrhotic in 12 (37.5%) cases and had lesions consistent with chronic hepatitis in eight (25%) cases; HBV infection was present in 12 cases, while HCV was reported in four cases and unconfirmed in 16 cases. The 37.5% of patients that tested positive for hepatitis B antigens did not test positive for anti-HCV antibodies.

The clinical and pathological features that were assessed included tumor size, histological grade, tumor stage, capsular and vascular invasion (invasion of small vessels and thrombi in the portal vein), satellite tumor nodules and serum alpha-fetoprotein (α-FP).

The size of hepatic tumors ranged from 1 cm to 7.5 cm and were classified as follows: 30 (93.75%) low-grade HCC and two (6.25%) high-grade HCC (six cases were grade I, 24 were grade II and two were grade III–IV).

HCC satellite tumor nodules were defined as the intra-hepatic extension of the tumor or as the multicenter origin of the tumor. DN were assessed as low grade in four cases and high grade in three cases.

E-cadherin expression in non-tumor liver

We considered as normal a uniform staining pattern of the hepatocyte membrane, whereas the uniform negative pattern and the heterogeneous pattern (cytoplasmic and membranous) were considered as an aberrant E-cadherin expression.

In normal hepatocytes, we observed a homogeneous, moderate/intense and diffuse E-cadherin immunoreaction at cell membrane level (in some cases also at cytoplasmic level), sometimes with the gradual decrease of staining intensity from tumor-free tissue to HCC.

Most hepatocytes of cirrhotic livers or chronic hepatitis lesions have shown a membrane pattern of E-cadherin staining that was intense and diffuse. In some cases, cirrhotic nodules had aberrant E-cadherin expression, with heterogeneous pattern and an increased membrane expression of hepatocytes located at the periphery of the nodules. In addition, we observed an intense staining with heterogeneous pattern of proliferated bile ducts from inter-nodular connective septa, crowded in the immediate vicinity of the regeneration nodules.

In all the analyzed cases, intra-hepatic bile ducts expressed an intense and homogeneous E-cadherin membrane reaction that was used as positive internal control of the reaction. Moreover, proliferated bile ducts expressed intensely E-cadherin at cellular membrane level, with a more intense staining than normal tumor-free hepatocytes. In one trabecular/sinusoidal HCC with extension to the gallbladder, we observed an intense membrane reaction to E-cadherin in the biliary-type epithelial cells.

E-cadherin expression in HCC and correlation with clinical and pathological features of patients

We observed: (*i*) negative E-cadherin reaction in 14 out of the 32 (43.75%) HCC cases; (*ii*) E-cadherin immuno-reactivity in all seven (100%) DN and 18 out of the 32 (56.25%) HCC cases; (*iii*) normal E-cadherin expression, with uniform membrane staining (Figure 1, A and B) in 6/32 (18.75%) cases and aberrant expression of E-cadherin (Figure 2, A and B) in 26/32 (81.25%) cases; (*iv*) hetero-geneous staining pattern (membrane and cytoplasm) in 12/26 (46.15%) HCC cases (Table 1).

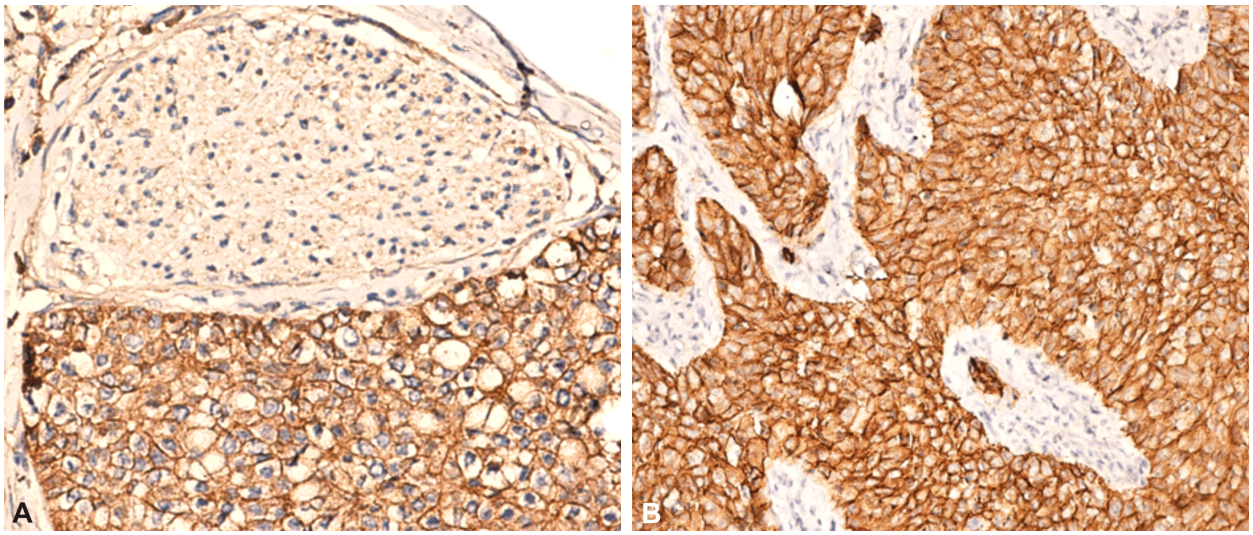

Figure 1 – ***(A and B) HCC with normal E-cadherin expression: uniform membrane staining pattern. Anti-E-cadherin antibody immunostaining, ×200. HCC: Hepatocellular carcinoma.***

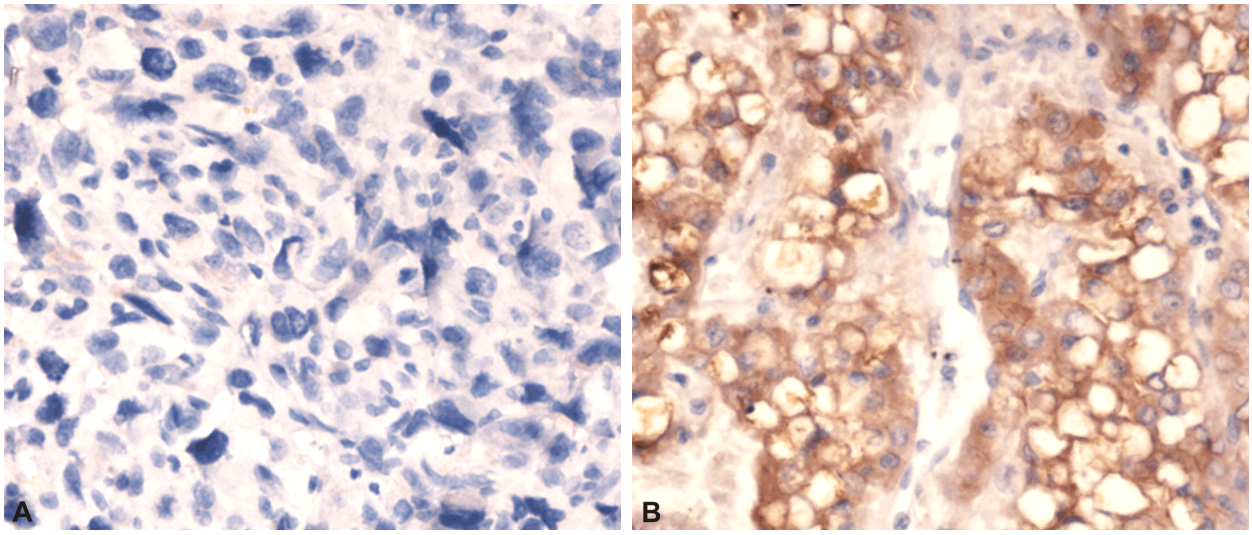

Figure 2 – ***HCC with aberrant E-cadherin expression: (A) Uniform negative pattern; (B) Membranous and cytoplasmic. Anti-E-cadherin antibody immunostaining, ×200. HCC: Hepatocellular carcinoma.***

Table 1 – ***Patterns of E-cadherin expression in HCC (n=32)***

Normal pattern (uniform membrane staining)	Aberrant E-cadherin expression	
	Heterogeneous pattern (membrane + cytoplasm)	*Uniform negative pattern*
6 (18.75%)	12 (46.15%)	14 (53.85%)
E-cadherin immunoreaction 18 (56.25%)		**Negative E-cadherin expression** 14 (43.75%)

HCC: Hepatocellular carcinoma.

Aberrant E-cadherin expression was observed more frequently in DN (p=0.285) and in HCC, as compared to the normal hepatic tissue surrounding the tumor (p<0.001) (Table 2).

Table 2 – ***E-cadherin expression in HCC associated lesions***

Lesion	Total case No.	E-cadherin expression		*p*-value (*Z* test)
		Normal (+) (%)	*Aberrant (± → -) (%)*	
Normal peritumoral liver	32	32 (100%)	0	<0.001
Chronic viral hepatitis	8	8 (100%)	0	<0.001
Liver cirrhosis	12	12 (100%)	0	<0.001
DN	7	2 (28.57%)	5 (71.43%)	0.285
HCC	32	6 (18.75%)	26 (81.25%)	<0.001

HCC: Hepatocellular carcinoma; DN: Dysplastic nodules.

Positive E-cadherin expression was observed in close percentages in men and women (58.33% and 55%, respectively) (p=0.854) (Table 3). We recorded 10 (50%) cases of HCC, with positive E-cadherin expression in patients aged 60 or below and eight (66.6%) cases of positive E-cadherin HCCs in patients above 60 years of age (p=0.581).

Table 3 – ***Correlation between E-cadherin expression and clinical and morphological parameters of patients***

Clinical and morphological parameters		Total case No. (*n*=32)	E-cadherin expression		*p*-value (χ^2 test)
			Positive (+ → ±) (%)	*Negative (-) (%)*	
Gender	*Men*	12 (37.5%)	7 (58.33%)	5 (41.66%)	0.854
	Women	20 (62.5%)	11 (55%)	9 (45%)	
Age [years]	*≤60*	20 (62.5%)	10 (50%)	10 (50%)	0.581
	>60	12 (37.5%)	8 (66.66%)	4 (33.33%)	
Tumor location	*RHL*	12 (37.5%)	8 (66.66%)	4 (66.66%)	0.429
	LHL	8 (25%)	3 (37.5%)	5 (62.5%)	
	Bilateral	12 (37.5%)	7 (58.33%)	5 (41.66%)	

RHL: Right hepatic lobe; LHL: Left hepatic lobe.

The results we obtained do not suggest a correlation between E-cadherin expression and tumor location, a positive E-cadherin reaction being recorded in eight (66.66%) of the cases with HCC in the right hepatic lobe (RHL), three (37.5%) cases of HCC located in the left hepatic lobe (LHL) and seven (58.33) cases of HCCs that developed bilaterally (p=0.429) (Figure 3).

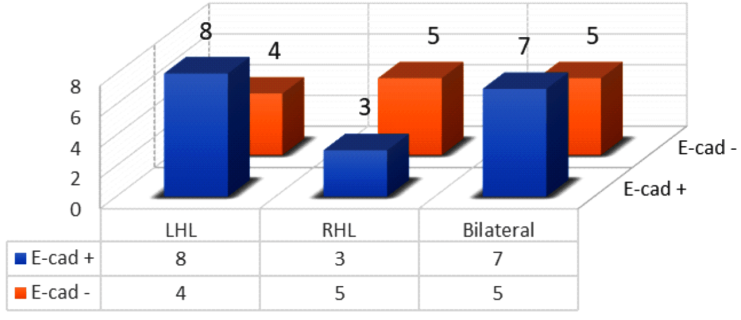

Figure 3 – ***Relationship between E-cadherin (E-cad) and tumor localization. LHL: Left hepatic lobe; RHL: Right hepatic lobe.***

We did not observe a correlation between E-cadherin expression and the presence of HBV or HCV infections; nine of the 12 (75%) HCCs with E-cadherin expression tested positive for HBV antigens, while only two out of four (50%) cases of HCC with positive E-cadherin expression tested positive for anti-HCV antibodies. E-cadherin was present in 75% of HCCs smaller than 5 cm and in 53.57% of HCCs with more than 5 cm (p=0.788) (Table 4).

E-cadherin assessment according to the histological type showed that positive E-cadherin reaction was more frequent in trabecular/sinusoidal (72.73%) (Figure 4) and acinar (75%) HCCs, as compared to the solid/pelioid type (33.33%) and the clear cell with bile secretion variant (40%) (p=0.15), which had an aberrant expression in over 50% of cases (Table 4). In tumor nodules with clear cells, we observed a more intense membrane E-cadherin expression in peripheral tumor cells. Also, we observed a mild cytoplasmic E-cadherin expression in pelioid HCCs and an intense reaction (in patches) in tumor areas with bile secretion. In two of the solid pattern HCCs with giant cells and fat secretion, we identified a heterogeneous moderate/intense staining pattern.

Table 4 – ***Correlation between E-cadherin expression and clinical and morphological parameters of patients***

Clinical and morphological parameters		Total case No. (n=32)	E-cadherin expression		p-value (χ^2 test)
			Positive (+ → ±) (%)	*Negative (-) (%)*	
Associated viral infection	*HBV*	12 (37.5%)	9 (75%)	3 (25%)	0.513
	HCV	4 (12.5%)	2 (50%)	2 (50%)	
	*	16 (50%)	9 (56.25%)	7 (43.75%)	
Tumor size [cm]	*<5*	4 (12.5%)	3 (75%)	1 (25%)	0.788
	>5	28 (87.5%)	15 (53.57%)	13 (46.43%)	
Histological type	*Trabecular*	11 (34.37%)	8 (72.73%)	3 (27.27%)	0.15
	Acinar	8 (25%)	6 (75%)	2 (25%)	
	Solid/pelioid	6 (17.75%)	2 (33.33%)	4 (66.66%)	
	Clear cells	5 (15.63%)	2 (40%)	3 (60%)	
	Anaplastic	2 (6.25%)	0 (0%)	2 (100%)	
Histological grade	*I+II*	30 (93.75%)	18 (60%)	12 (40%)	0.358
	III+IV	2 (6.25%)	0 (0%)	2 (100%)	

HBV: Hepatitis B virus; HCV: Hepatitis C virus.

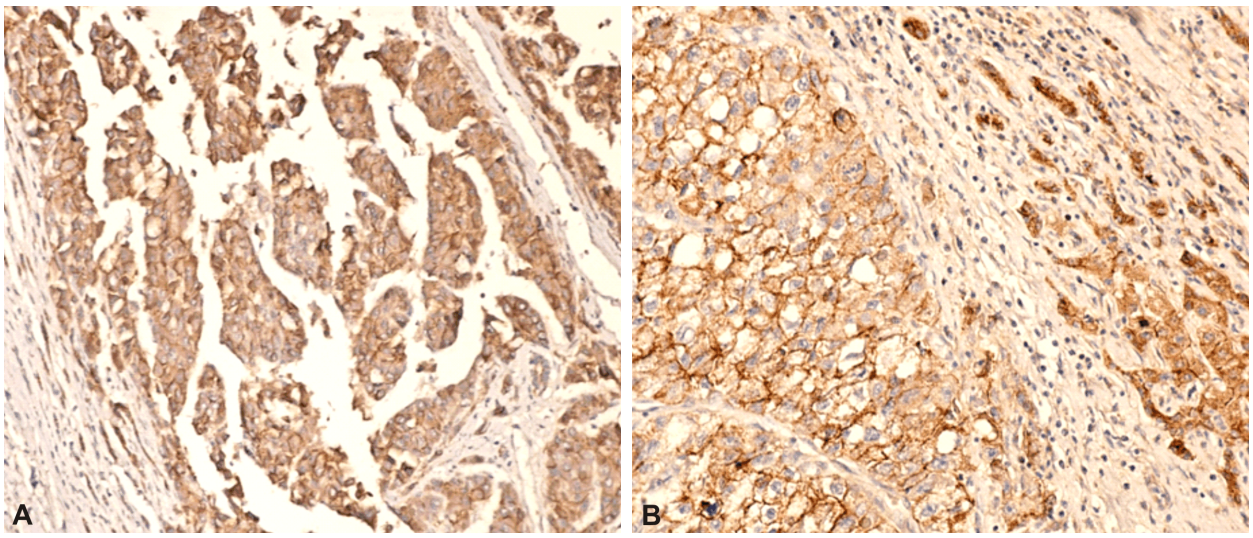

Figure 4 – ***HCC with membranous E-cadherin expression: (A) Trabecular/sinusoidal; (B) With clear cells and fat deposits. Anti-E-cadherin antibody immunostaining, ×200. HCC: Hepatocellular carcinoma.***

Low and high-grade DN within HCCs developed on a cirrhotic background expressed E-cadherin with a predominantly membrane staining pattern (Figure 5). Poorly differentiated and anaplastic HCCs with giant or spindle cells did not express E-cadherin (Figure 6).

In low-grade HCCs (Edmondson & Steiner I+II), we found E-cadherin expression in 18 (60%) cases and a negative reaction in 12 (40%) cases of HCCs. In both high-grade (III+IV) HCCs, we found that the E-cadherin reaction was completely absent (p=0.358).

The rate of positive E-cadherin reaction was significantly higher in tumors associated with capsule infiltration (15/20; 75%) (p=0.017) and does not seem to be influenced by the vascular invasion (15/24; 62.5%) (p=0.411), the presence of satellite nodules (p=0.285) or the serum level of α-FP (p=0.787) (Table 5).

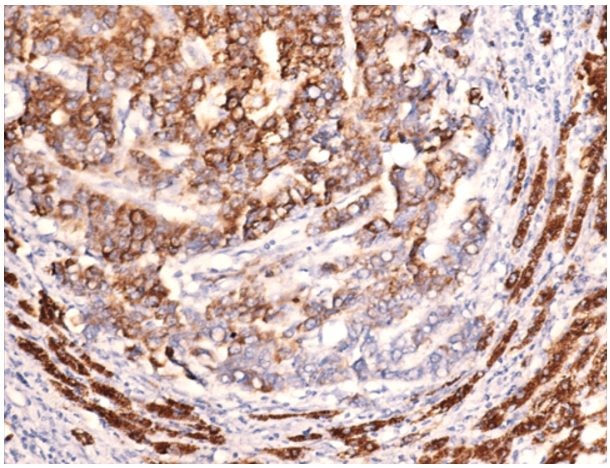

Figure 5 – ***E-cadherin expression in high-grade DN. Anti-E-cadherin antibody immunostaining, ×200. DN: Dysplastic nodules.***

Discussions

HCC is the most common hepatic primary malignant tumor and the fifth most frequent amongst malignant tumors, with 372 000 new cases diagnosed every year [31]. Two hypotheses concerning the pathogenesis of HCC were developed: *de novo* hepatic carcinogenesis and step-by-step hepatic carcinogenesis.

DN, described as precursor lesions in hepatic step-by-step carcinogenesis, evolve into DN with microscopic HCC foci and eventually into HCC, in some cases with the possibility of metastasis. Few known studies analyze the genetic and epigenetic alterations of E-cadherin in DN [25]. Despite recent developments regarding diagnostic and therapeutic methods, long-term survival of patients with HCC is still reduced because of the high recurrence rate after initial treatment.

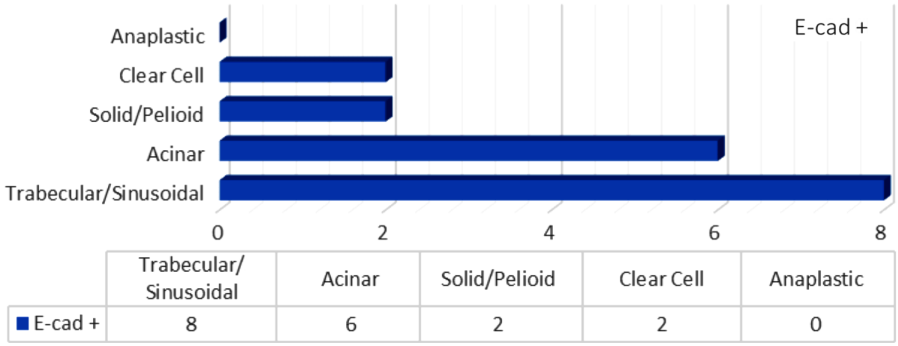

Figure 6 – ***Relationship between E-cadherin (E-cad) and histological type.***

Table 5 – ***Correlation between E-cadherin expression and clinical and morphological parameters of patients***

Clinical and morphological parameters		Total case No. (*n*=32)	E-cadherin expression		*p*-value (χ^2 test)
			Positive (+ → ±) (%)	*Negative (-) (%)*	
Capsule infiltration	*Present*	20 (62.5%)	15 (75%)	5 (25%)	0.017
	Absent	12 (37.5%)	3 (25%)	9 (75%)	
Satellite nodules	*Present*	16 (50%)	7 (43.75%)	9 (56.25%)	0.285
	Absent	16 (50%)	11 (68.75%)	5 (31.25%)	
Vascular invasion	*Present*	24 (75%)	15 (62.5%)	9 (37.5%)	0.411
	Absent	8 (25%)	3 (37.5%)	5 (62.5%)	
α-FP [ng/mL]	*≤400*	14 (43.75%)	8 (57.14%)	6 (42.86%)	0.787
	>400	18 (56.25%)	10 (55.55%)	8 (44.44%)	

α-FP: Alpha-fetoprotein.

Cadherins are transmembrane glycoproteins and primary mediators of calcium-dependent cellular adhesion, members of this family being found in various locations: E-cadherin in epithelial tissue, N-cadherin in muscle and adult neural tissue and P-cadherin in the placenta [32].

E-cadherin and β-catenin have an active role in the regulation of epithelial cell adhesion, being closely related to cancer invasiveness and β-catenin dystopian expression in early stage HCC [33].

E-cadherin is a transmembrane glycoprotein that plays an important role in the contact between calcium-dependent epithelial cells. Cellular adhesion mediated by the interaction of the extracellular domain of E-cadherin is involved in establishing cell polarity. The cytoplasmic domain of E-cadherin is linked to the actin cytoskeleton *via* catenins (α-, β-, γ-catenin and p120). The E-cadherin/β-catenin complex is known as a suppressor for tumors and metastases, metastasis repression strategies leading to the study of mechanisms and molecules that regulate E-cadherin function [34].

E-cadherin plays a key role in maintaining epithelial integrity and cellular polarity, normal E-cadherin immuno-expression being localized in the cell membrane [35, 36].

Somatic mutations of E-cadherin were identified in poorly differentiated breast and gastric cancer, but have not yet been described in HCC. In hepatic cancer, allelic deletion of E-cadherin [24], E-cadherin gene methylation or mutation of exon 3 of β-catenin have been reported,

significant correlations being identified between them and tumor development/progression [37, 38].

Currently, there are few studies demonstrating the relationship between the co-expression of the five cell adhesion molecules mentioned above and the clinical-pathological parameters, including the prognosis of patients with HCC.

E-cadherin and β-catenin expression were inversely correlated with the degree of tumor differentiation, being considered useful markers for HCC differentiation. Endo *et al.* (2000) [39] identified α-, β- and γ-catenin over-expression, inversely proportional to the histological grade in most analyzed HCCs and a significant positive correlation between strong β-catenin expression and vascular invasion. Endo *et al.* (2000) [39] and Ihara *et al.* (1996) [40] described the overexpression of E-cadherin and β-catenin in most HCCs, E-cadherin being able to act as a modulator in maintaining the histological architecture of HCC.

Analyzing the prognostic value of E-cadherin expression in 91 operated HCCs, Garcia *et al.* (1998) [41] noted the alteration of E-cadherin expression in 56% of tumors, correlated with the morphological parameters with prognostic value, such as tumor size (>3 cm), nuclear grade and high mitotic index. The survival rate was significantly reduced in patients with low E-cadherin expression.

In our study, we analyzed IHC expression of E-cadherin in 32 HCCs and adjacent non-tumor liver tissue using the monoclonal anti-E-cadherin antibody, clone EP700Y. Non-tumor hepatocytes, bile duct epithelium and proliferating canals strongly expressed E-cadherin at cell membrane level (in some cases also in the cytoplasm), but the intensity of staining decreased from non-tumor tissues towards the tumor cells.

We identified a strong and diffuse membrane-staining pattern with E-cadherin in non-tumor hepatocytes from cirrhotic liver or with chronic active hepatitis lesions and an aberrant expression of E-cadherin, with a heterogeneous pattern and increased membrane expression in hepatocytes at the periphery of cirrhotic nodules. Proliferating bile ducts from the inter-nodular connective septa and those crowded in the immediate vicinity of regeneration nodules had a strong E-cadherin staining with heterogeneous pattern.

Overall, intrahepatic biliary ducts expressed a strong and homogeneous membranous E-cadherin reaction, serving as positive internal control. Proliferated bile ducts showed strong membrane immunoreaction to E-cadherin, the staining being more intense than that of non-tumor hepatocytes.

We found normal E-cadherin expression with uniform membrane staining in 18.75% of HCCs and aberrant expression in 81.25% of cases; in 46.15% of HCCs, we identified a heterogeneous staining pattern (membranous and cytoplasmic). We noted negative E-cadherin immuno-staining in 43.75% of HCCs and positive E-cadherin immunoreaction in all seven DN and 56.25% of HCCs.

The results obtained show: (*i*) aberrant expression of E-cadherin more frequently in DN and hepatic carcinomas, as compared to the normal liver tissue surrounding the tumor and (*ii*) positive E-cadherin expression in similar proportions in men and women.

We did not find a statistically significant relationship between E-cadherin expression and tumor localization, presence of viral infection (with HBV or HCV) or tumor size.

Assessing E-cadherin expression according to the histological type of HCC, we identified positive E-cadherin immunoreaction more commonly in trabecular/sinusoidal and acinar HCCs, as compared to solid/pelioid HCCs or to HCCs with clear cells and bile secretion; however, these results were statistically not significant. In clear cell HCC, we observed a more intense E-cadherin membrane expression in tumor hepatocytes at the periphery of the nodules. Pelioid-type HCCs exhibited weak cytoplasmic E-cadherin immunostaining, with strong membrane expression in areas with bile secretion. We identified a moderate/intense heterogeneous E-cadherin staining pattern in two cases of solid HCCs, with giant cells and fat secretion, a predominantly membranous staining pattern in DN associated with carcinomas developed on a cirrhotic background and the absence of E-cadherin expression in poorly differentiated/anaplastic carcinomas.

Ihara *et al.* (1996) [40] noted the overexpression of E-cadherin, inversely proportional to the histological grade of the tumor in all 66 HCCs studied cases. We found positive E-cadherin expression in 60% of low grade HCC (Edmondson & Steiner I+II) and negative immunoreaction in 40% of cases. High-grade HCCs (III+IV) were not immunoreactive to E-cadherin.

Kozyraki *et al.* (1996) [42] reported an association between E-cadherin expression in HCC and vascular and capsular invasion. In our study, positive expression of E-cadherin was significantly higher in tumors associated with capsule infiltration (15/20; 75%) (p=0.017), but it was not influenced by vascular invasion (15/24; 62.5%) (p=0.411), the presence of satellite tumor nodules or α-FP serum levels.

The meta-analysis carried out by Chen *et al.* [43] demonstrated that reduced expression of E-cadherin was correlated with reduced survival at one, three and five years, respectively, in patients with HCC. In addition, it was significantly correlated with the presence of metastases and vascular invasion, low degree of differentiation, as well as with advanced tumor, node, metastasis (TNM) stages. Therefore, E-cadherin expression appears to have predictive potential for patients diagnosed with HCC.

Conclusions

Aberrant expression of E-cadherin is significantly observed in DN and HCCs, as compared to the normal liver tissue surrounding the tumor. The results we obtained suggest the important role of E-cadherin in the development of differentiated forms of HCCs, E-cadherin being considered a marker of histological differentiation. E-cadherin expression is an important prognostic factor, correlated with histological grade, but not with patients' age, HBV or HCV infection or tumor size. The rate of

E-cadherin positive expression is significantly higher in HCCs associated with capsule infiltration and is not influenced by vascular invasion, presence of satellite nodules or α-FP serum levels.

Conflict of interests

The authors have no conflict of interests to report.

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