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E-cadherin and β-catenin immunoexpression in endometrioid endometrial carcinoma

MIRELA MARINELA FLORESCU[1)], DANIEL PIRICI[2)], CRISTIANA EUGENIA SIMIONESCU[1)], ALEX EMILIAN STEPAN[1)], CLAUDIU MĂRGĂRITESCU[1)], ŞTEFANIA TUDORACHE[3)], RALUCA NICULINA CIUREA[1)]

[1)]*Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Research Methodology, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

E-cadherin and β-catenin are two molecules implicated in cellular adhesion. The reduced expression of β-catenin and E-cadherin is associated with the tumoral epithelial-mesenchymal transition process, a key event in the development of endometrial carcinoma. The aim of our study was to investigate the association between the immunoexpression of β-catenin/E-cadherin and the tumor differentiation degree, presence of lymph nodes, depth of tumor invasion and pTNM stage of endometrioid endometrial carcinomas in order to enhance the potential aggressiveness of these tumors. Our results revealed significant differences in the expression of β-catenin, when grouping for the tumor stage, invasion in the myometrium and degree of differentiation, as well as significant differences in the expression of E-cadherin for tumor degrees of differentiation. E-cadherin and β-catenin expression was stronger in well-differentiated tumors, superficial myometrium invasion and early tumor stages I or II, thus was associated with better prognostic forms of endometrioid endometrial carcinoma. Our study indicated that decreased of the E-cadherin and β-catenin expression is useful for the assessment of tumor aggressivity.

Keywords: E-cadherin, β-catenin, endometrioid endometrial carcinoma.

Introduction

Endometrial cancer is responsible for approximately 4% of all cancers that affect women worldwide, especially after menopause [1]. The aggressiveness of these tumors can be explained by the intervention of the epithelial-mesenchymal transition process.

One of the features of epithelial-mesenchymal transition process is the loss of intercellular adhesion that is associated with lower E-cadherin expression [2, 3]. E-cadherin is a transmembrane protein with five extracellular domains and an intracellular domain that connects the actin cytoskeleton by a cytoplasmic catenin complex. The decrease of E-cadherin expression is associated with a loss of cell–cell adhesion, which was proved by tumor cell motility changes, and it is a characteristic of tumor cell lines with high metastatic potential [4]. Decreased expression of E-cadherin is found in about 5–40% of endometrioid carcinomas [5]. Tumors without E-cadherin expression are more likely to be poorly differentiated or non-endometrioid and are often associated with a worse prognosis [5].

Another protein involved in the cellular adhesion is β-catenin, which is reportedly implicated in endometrial and ovarian carcinogenesis [6]. β-Catenin was first described as a component of the cadherin/catenin complexes mediating calcium-dependent intercellular adhesion [7]. Relying on it cellular localization β-catenin has many roles besides cellular adhesion. β-Catenin is a key element of the Wnt signaling pathway, which is linked to multiple cellular processes such as proliferation, migration and differentiation that are involved in the development of the embryo and adult homeostasis. Dysfunction in the activation of the Wnt/β-catenin pathway can promote cancer development [8]. Furthermore, β-catenin gene (CTNNB1) mutations can lead to decreased cell–cell adhesion and have been reported in about 15% of endometrioid carcinomas [9, 10].

Aim

The aim of our study was to investigate the association between the immunoexpression of β-catenin, E-cadherin and the tumor degree of differentiation, presence of lymph nodes, depth of tumor invasion and pTNM stage of endometrioid endometrial carcinomas in order to enhance the potential aggressiveness of the tumors.

Patients and Methods

The study included a total of 40 patients hospitalized during 2011–2014 in the Clinics of Obstetrics, Gynecology and Surgery, Emergency County Hospital of Craiova, Romania. The 40 cases in our study were represented by total hysterectomy specimens, which were analyzed and diagnosed in the Department of Pathology of the same Hospital, where the specimens were fixed in 10% neutral buffered formalin, processed by paraffin embedding and Hematoxylin–Eosin (HE) staining. Clinical and morphological parameters investigated were age, differentiation degree, lymph node status, depth of invasion, pTNM stage. For the classification and analysis of the lesions, we used the criteria established by *World Health Organization* (WHO) in 2014 [11]. In this study were included only endometrioid endometrial carcinomas, without distant metastases. The study was approved by the local ethical committee and a written informed consent was obtained

from all the patients. For immunohistochemical analysis, we used a panel of antibodies, as showed in Table 1.

Table 1 – ***The antibodies we used in the study***

Antibody	Host, clone, manufacturer	Dilution	Pretreatment	External positive control
β-Catenin	Mouse anti-human / β-catenin-1 / Dako	1:100	Microwaving in citrate buffer, pH 6	Liver
E-cadherin	Mouse anti-human / NCH38 / Dako	1:50	Microwaving in citrate buffer, pH 6	Mammary gland

The amplification system was represented by LSAB2 System–HRP (Horseradish peroxidase) (DAKO, Redox, Bucharest, Romania, code K0675) and the signal visualization was done with 3.3'-diaminobenzidine tetrahydrochloride (DAB, Dako, code 3467). For the statistical analysis, there were used Student's *t*-test, one-way ANOVA, and Pearson's comparative and correlation tests within SPSS 17 software. Average values are reported ± standard deviation (SD). Image acquisition was performed using a Nikon Eclipse E600 microscope and the Lucia 5 imaging software. Results were considered significant for *p*-values <0.05. Quantification of the immunostainings was done in parallel by two of the authors (FM, SA), and the final values were tested for kappa concordance index (Cohen's kappa coefficient). The antibodies quantification has been performed by using a score resulted through multiplying the number of marked cells (P) with the immunostaining intensity (I). Thus, according to the number of the marked tumor cells, the studied cases were divided into the following categories: 0 (the absence of marked cells), 1 (<10% marked cells), 2 (10–25% marked cells), 3 (25–50% marked cells), and 4 (>50% marked cells). The intensity of the marked cells was divided in four categories: 0 (absent), 1 (poor), 2 (moderate) and 3 (strong). For the statistical analysis, the resulting scores were considered low for values between one and four and high for values between 6 and 12.

Results

From the 40 patients, the analysis of morphological parameters indicated an average age at diagnosis of 60.8 years (Table 2). Most of the analyzed endometrial carcinomas were well and moderate differentiated (19, respectively 12 cases), with invasion into the internal half of myometrium (23 cases) and without lymph node metastases (38 cases). Also, the majority of the cases were classified in the pTNM stage I of disease (23 cases). In our study, the number of cases stratified for the depth of invasion and tumor stage was the same (Table 2).

E-cadherin immunostaining was identified in the tumor cells membrane in 85% of cases. E-cadherin intensity and percentage immunostained cells were different depending on the differentiation degree. Well-differentiated carcinomas indicated an average marked cells of 69.6±31.4, the intensity reaction was strong/moderate, with an average score of 8.3. In comparison, moderately and poorly differentiated carcinomas revealed a mean percent value of 53±36.1 and 30.5±30.2, respectively. The intensity of the reactions was also variable, and the mean scores were 5.2 and 2.8 (Table 3; Figure 1, A–C).

Table 2 – ***Cases distribution depending on clinical and morphological parameters***

Parameter	Variable and No. of cases
Age [years]	<50: 3
	>50: 37
Differentiation degree	WD: 19
	MD: 12
	PD: 9
Lymph node metastasis	N0: 38
	N1: 2
Depth of invasion/stage	T1/stage I: 23
	T2/stage II: 12
	T3/stage III: 5

WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated.

Table 3 – ***Immunostaining medium values in relation with clinical and morphological parameters***

Parameter	Variable and No. of cases	E-cadherin [%]		β-Catenin [%]	
			Score		*Score*
Age [years]	<50: 3	96.3± 6.3	9.3	59.3± 52.1	5.3
	>50: 37	54.9± 34.1	6.1	59.2± 37.2	6.2
		p=0.145		*p*=0.339	
Differentiation degree	WD: 19	69.6± 31.4	8.3	69± 38.4	7.3
	MD: 12	53± 36.1	5.2	61.2± 34.4	6.7
	PD: 9	30.5± 30.2	2.8	36.1± 33.61	3
		p=0.004		*p*=0.019	
Lymph node metastasis	N0: 38	67.3± 26.1	7.5	72.3± 28.55	7.1
	N1: 2	50± 70.7	4	28± 39.5	6
		p=0.413		*p*=0.417	
Depth of invasion/ stage	T1/stage I: 23	59.5± 33.3	7.3	64.9± 35	6.4
	T2/stage II: 12	65.3± 29.6	5.8	63.1± 39.1	6.9
	T3/stage III: 5	34± 47.7	3.2	40.2± 37.6	4.8
		p=0.229		*p*=0.025	

WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated.

In relation to the depth of invasion and tumor stage, the average percentages of the marked tumor cells were higher in pT1/stage I and pT2/stage II (59.5±33.3, 65.3± 29.6) and the intensity of the reaction was variable, with an average score of 7.3 and 5.8, respectively. By contrast, in pT3/stage III the average percentage of labeled tumor cells was of 34±47.7, with the intensity of the reaction variable with an average score of 3.2.

The β-catenin immunostaining was identified in the apical cytoplasm and membrane of tumor cells in 85% of the cases. β-Catenin immunostaining for endometrioid endometrial carcinoma also varied depending on the degree of tumor differentiation. Well-differentiated carcinomas showed an average of marked cells of 69±38.4, the intensity of the reaction was variable and the average score was 7.3. In comparison, moderately and poorly differentiated carcinomas had mean percent values of 61.2±34.4 and 36.1±33.61. The intensity of the reactions was also variable and the mean scores were of 6.7 and respectively 3 (Table 3; Figure 1, D–F).

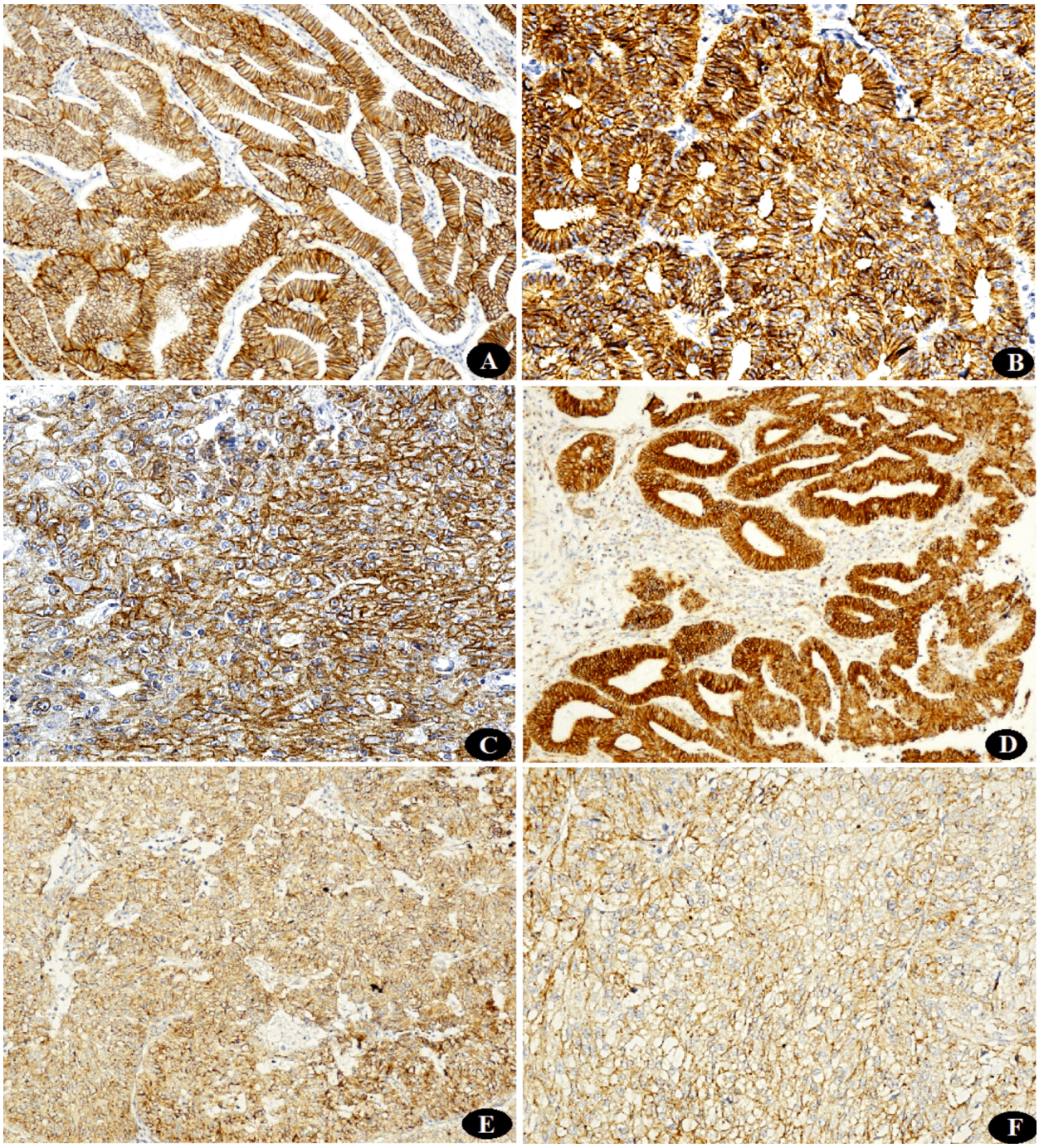

Figure 1 – ***Endometrioid endometrial carcinoma, E-cadherin (A–C) and β-catenin (D–F) immunostaining, 100×: (A and D) Well differentiated; (B and E) Moderate differentiated; (C and F) Poorly differentiated.***

When considering the depth of invasion and tumor stage, the average percentage of the β-catenin marked tumor cells was also higher in pT1/stage I (64.9±35), the intensity of the reaction was variable, and the average score was of 6.4 compared with pT2/stage II and pT3/stage III, where the average percentage of marked tumor cells were of 63.1±39.1 and 40.2±37.6. The intensity of the reaction was also variable with a mean score of 6.9 or 4.8.

Regarding the histopathological parameters analyzed, we have found significant differences in the expression of E-cadherin in relation to the degree of differentiation (p=0.004, ANOVA test) (Table 3, Figure 2A). However, our results did not certify significant differences between the expression of E-cadherin in relation with the invasion in the myometrium and the tumor stage, even though we documented higher expression of E-cadherin in stages I and II in contrast with stage III.

Our study indicates significant differences for the β-catenin expression and degree of differentiation (p=0.019, ANOVA test), the invasion in the myometrium (p=0.025, ANOVA test) and the tumor stage (p=0.034, ANOVA test) (Table 3; Figure 2, B and C).

We have not found significant differences in the expression of E-cadherin and β-catenin in relation to pN category, although values for both reactions were higher in carcinomas without lymph node metastases. In addition, analysis of the percentages of E-cadherin and β-catenin showed a positive linear correlation (p=0.001, Pearson's test) (Figure 2D).

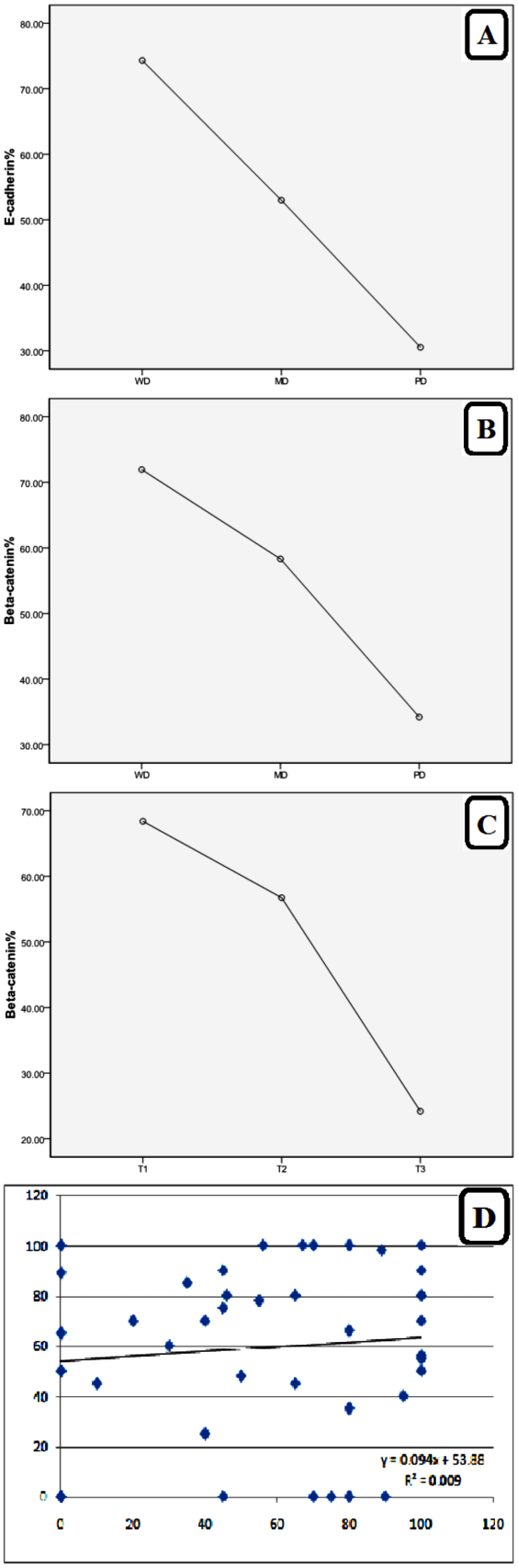

Figure 2 – ***ANOVA graphic representation of E-cadherin statistical differences regarding the tumor differentiation degree (A) and β-catenin regarding the tumor differentiation degree (B) and depth of invasion (C); WD: Well differentiated, MD: Moderate differentiated, PD: Poorly differentiated. (D) Scatterplot correlation graphic representation for E-cadherin and β-catenin.***

Discussion

E-cadherin and *β*-catenin are two molecules involved in cellular adhesion. The loss of intercellular adhesion, associated with reduced expressions of *β*-catenin and E-cadherin is an element of the epithelial-mesenchymal transition, which is a key process in the development of endometrial carcinoma. Furthermore, *β*-catenin is involved in signaling the Wnt/*β*-catenin transduction pathway, which is reported to contribute even more in the process of carcinogenesis [6]. The expression of these two molecules has been studied in relation with various clinical and pathological aspects in cancers including endometrioid endometrial carcinomas.

We have studied here the expression of E-cadherin and *β*-catenin in endometrioid endometrial carcinomas in patients with the ages, at the moment of diagnosis, varying from 36 to 92 years old, most cases belonging to the age group of 60–79 years. The literature also recognizes that type I endometrial cancers are often diagnosed at an age between 59 and 67 years [12]. Furthermore, Zusterzeel *et al.* claimed that a diagnosis age of 60 years old represents a relevant prognostic factor linked with a high risk of recurrence, even though endometrial cancers can be diagnosed at an early age [13, 14]. Our results regarding the expression of E-cadherin revealed significant differences with the tumor degree of differentiation. On the other hand, E-cadherin expression was stronger in well-differentiated tumors, superficial myometrium invasion and early tumor stages, thus associating with better prognostic of endometrioid type of endometrial carcinoma. The expression of E-cadherin was analyzed by Ahmed & Muhammad, in relation with the expression of CD10, in 28 cases of endometrial carcinomas, 19 cases of endometrial hyperplasia and seven cases of normal endometrial changes. The study reported a high expression of E-cadherin in endometrial carcinomas, and similar to our findings, the expression of E-cadherin was lower in high-grade tumors. Therefore, the expression of both CD10 and E-cadherin displayed no association with the depth of tumor invasion and FIGO (*International Federation of Gynecology and Obstetrics*) stage. The authors concluded that lower expression of E-cadherin and CD10 are important for endometrioid endometrial carcinomas progression [15]. Another study assessed the expression of several markers such as E-cadherin, *α*-catenin, N-cadherin, vimentin in relation with the overexpression of epidermal growth factor (EGFR). The results suggested that the expression of E-cadherin was lower in higher tumor stages of endometrial carcinoma tissues [16].

E-cadherin has been reported to also offer a predictive outcome value in the management of endometrioid endometrial carcinomas and for this reason, a recent study determined the outcome implications of epithelial-mesenchymal transition-related proteins (E-cadherin and Snail) in relation to HIF-1*α* in endometrioid endometrial carcinomas (EECs). The results implied that E-cadherin expression levels were correlated with histopathological grade, myometrial invasion, and lymph node metastasis. Therefore, the study suggested that although Snail and HIF-1*α* expressions were significantly correlated with a poor outcome in patients with EEC, the expression of E-cadherin is acknowledged as favorable and together with the Snail expression can offer a predictive outcome

value in the management of the studied carcinomas [17, 18]. González-Rodilla *et al.* tried to prove a paradox that although E-cadherin is independently associated with good survivability, the co-expression of E-cadherin with various molecular markers of proliferation such as Ki67, human epidermal growth factor or p53 was associated with poor prognosis [19].

Our results also certified significant differences in the expression of β-catenin in relation with tumor stage, invasion in the myometrium and degree of differentiation.

In a similar manner to our results, other studies suggest that positive β-catenin expression in patients with endometrial carcinoma is significantly associated with decreased tumor stage and histological grade [20]. Furthermore, some studies imply that the expression of β-catenin does also associate with negative lymph node tumor invasion, whereas E-cadherin expression was highly associated with lymph node invasion [21, 22].

Several studies have also assessed the associations between the expression of these markers and other clinicopathological aspects in various illnesses. Expression levels of both E-cadherin and β-catenin as well as N-cadherin were reported to be associated with tumor stage, tumor degree of differentiation and with lymph node metastasis in laryngeal carcinomas [23]. On the other hand, in hepatocellular carcinoma, the elevated expression levels of β-catenin were correlated with higher tumor degree, therefore suggesting β-catenin involvement in the development of metastasis [24, 25].

Furthermore, the expression of E-cadherin was also assessed in patients with pancreatic disorders and several studies concluded that the loss of E-cadherin expression for patients who suffered surgical resection by undergoing pancreaticoduodenectomy for pancreatic ductal adenocarcinoma is associated with poor prognostic. β-Catenin expression was also investigated in other types of cancers such as ovarian cancers, though less commonly then in endometrial cancers but also with prognostic value. Another study assessed β-catenin expression in synchronous tumors of the ovary or the endometrium and suggested that active mutations of the marker associated with different expression levels and offers the possibility to separate primary from metastatic tumors. In addition, β-catenin expression was highly associated with both tumor differentiation and FIGO staging in the ovarian carcinoma samples [8, 26].

Conclusions

In this study, the expression E-cadherin and β-catenin was superior in well-differentiated endometrioid endometrial carcinomas with superficial invasion and no lymph node metastasis, which allowed us to underline a positive linear correlation between the two markers. The alteration of intercellular adhesion system and the activation of transcriptional processes during the epithelial-mesenchymal transition in endometrial carcinomas are characteristic to aggressive tumors. Further studies should provide data concerning the mechanisms involved in the interaction of these markers with intra- or extracellular environment in order to fully understand the epithelial-mesenchymal transition process or to assess prognosis and possible future therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Mirela Marinela Florescu and Daniel Pirici equally contributed to the manuscript.

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Corresponding author
Ştefania Tudorache, Lecturer, MD, PhD, Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200628 Craiova, Romania; Phone/Fax +40251–599 228, e-mail: stefania.tudorache@gmail.com

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