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Immunohistochemical expression of E-Cadherin and β -catenin in prostate adenocarcinoma and benign prostate hyperplasia**Aysegul Isal Arslan¹, Sevil Karabag¹, Murat Akgul², Ilker Yildirim³**¹Tekirdag Namik Kemal University Faculty of Medicine, Department of Pathology, Tekirdag, Turkey²Tekirdag Namik Kemal University Faculty of Medicine, Department of Urology, Tekirdag, Turkey³Tekirdag Namik Kemal University Faculty of Medicine, Department of Anesthesiology and Reanimation, Tekirdag, Turkey

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Abstract

Disruption of the E cadherin mediated complex due to loss or depletion of E cadherin results in epithelial abnormalities and serious developmental impairment in various tissues and organs. The present study aims to determine E cadherin and β catenin expression in patients diagnosed with benign prostatic hyperplasia (BPH) and prostate carcinoma (PCa) based on Gleason scores and investigate the association of these proteins with PSA levels and Gleason scoring. Immunohistochemical staining for E cadherin and β catenin was performed in 59 patients diagnosed with PCa and 30 patients with BPH. Mean E cadherin expression was 3.00 in patients diagnosed with BPH and 2.38 ± 0.5 in patients with PCa, with a statistically significant difference between these values ($p < 0.001$). Comparison of PCa cases with PSA < 10 versus those with PSA ≥ 10 revealed significantly reduced β catenin expression in the group with PSA levels ≥ 10 ($p < 0.001$). Loss of E cadherin and β catenin is known to contribute to the pathogenesis of PCa. We believe that future molecular studies on this subject may further elucidate the association between carcinoma development and the expression of these molecules, leading to new therapeutic targets in the treatment of PCa.

Keywords: Prostate carcinoma, benign prostatic hyperplasia, E cadherin, β catenin**Introduction**

Changes in prostate gland occur with increasing age in all men. Prostate cancer (PCa) is the 2nd most common cancer in men worldwide (13%) and the 5th most common cause of cancer related mortality (6.7%) based on GLOBOCAN data [1]. Benign prostatic hyperplasia (BPH) on the other hand, is the most common cause of urinary dysfunction in elderly males (older than 60 years of age). Etiopathogenesis of PCa and BPH involves a range of factors that include age, ethnic origin, family history, diet, genetics and environmental influences [2-4]. Tumor growth is known to be associated with high levels of clinical and molecular heterogeneity, and molecules such as E cadherin, β catenin and Cox-2 are involved in carcinogenesis [5,6].

Cell to cell contact is essential for the development of multicellular organisms. Cadherins are key regulators in this biological process and play a critical role in morphogenic pathways during development [7]. E cadherin is the main member of the cadherin superfamily consisting of cytoplasmic catenin molecules and the actin cytoskeleton which maintain the integrity and functional characteristics of epithelium.

E cadherin is expressed on the surface of all epithelial cells [8]. E cadherin is a transmembrane cell adhesion molecule required to maintain normal cellular morphology and epithelial differentiation [9]. Loss of E cadherin is associated with epithelial-mesenchymal transition, a reversible alteration in cellular phenotype which allows cell migration and invasiveness [10,11]. Epithelial-mesenchymal transition (EMT) is an important step in tumor growth where invasive malignant cells are transformed from epithelial to mesenchymal phenotype. During this process, a reduction or loss occurs in the expression of certain adhesion molecules such as E cadherin required for the regulation of cell to cell adhesion, EMT, cancer cell migration and tissue invasion. EMT is of critical importance for the formation of invasive and metastatic phenotypes in prostatic carcinoma cells [6].

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β catenin is a protein involved in cell signaling and cellular adhesion that is found in the nucleus, membrane and the cytoplasm. Under normal circumstances, cellular β catenin levels are strictly regulated and abnormally increased β catenin expression has a confirmed association with oncogenic transformation during tumor development [12]. Increased nuclear β catenin levels resulting from mutations in β catenin and components of the degradation complex are associated with tumor formation and observed in various tumors [12,13].

E cadherin may control the location and protein levels of β catenin [14]. The membrane located E cadherin and β catenin complex is required for epithelial cell adhesion and also important for the formation and function of the prostate gland [10].

Lack of E cadherin expression in cell membrane leads to β catenin degradation, followed by relocation into the cytoplasm/nucleus. These events are associated with tumor invasiveness and metastasis both in dogs and humans [15,16]. Expression level of E cadherin shows a negative correlation with EMT and tumor invasion. Loss or excessive expression of E cadherin is associated with PCa progression, metastasis and poor prognosis through two distinct mechanisms, namely cell to cell adhesion and paracrine effect [17]. Furthermore, temporary deletion of E cadherin in cells that produce epithelial cells in prostate induces apoptotic cell death, promoting vertical divisions from basal prostate cells to luminal cells, thereby increasing luminal cell growth and expansion [18].

In this study, we aimed to determine E cadherin and β catenin expression in patients with BPH and in patients with PCa across different risk groups and investigate the association of these proteins with PSA levels, tumor growth and/or Gleason scoring, i.e. the main prognostic marker in this setting.

Materials and Methods

The present study included TUR-P (transurethral resection of the prostate) materials from 30 patients with BPH and radical prostatectomy materials from 59 patients with PCa diagnosed at Namık Kemal University Faculty of Medicine in 2012-2019. Age, PSA levels, histopathology results, risk groups, Gleason scores, presence of lymphovascular invasion, presence of perineural invasion and staging of the patients were recorded. A total of 30 TUR-P materials and 59 radical prostatectomy materials were extracted from the archive, concurrently re-evaluated by two pathologists and eligible tumor tissues and hyperplastic prostate tissues were selected for immunohistochemical staining for E cadherin and β catenin in paraffin blocks. Sections of 4 micron thickness were obtained from 89 formalin fixed, paraffin embedded tissues for the immunohistochemical assay and positive charged microscope slides were used to avoid tissue shedding. The sections were allowed in an incubator at 60°C for an hour and deparaffinized with xylol for 15 minutes. The samples were dehydrated through descending grade series of alcohol and rinsed in distilled water. Samples were then introduced to a BenchMark XT device. E cadherin (cell marque, RTU) and β catenin (cell marque, RTU) antibodies were applied and staining was performed subsequently. The samples stained in the automated staining device were covered with a fluid based covering material. A total of 100 cells in 5 separate fields were counted per case in the slides stained for E cadherin and β catenin and these cells were

scored as follows: score 1, incomplete membranous staining up to 25%; score 2, complete membranous staining of 25-50%; score 3, complete membranous staining >50% (Figure 1).

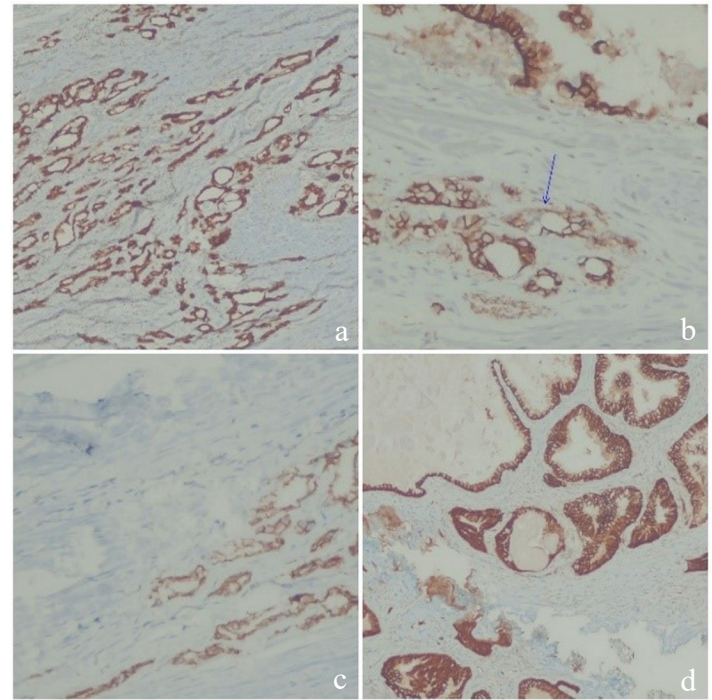


Figure 1 a) PCa, E-cadherin score3 x200 b) PCa, β catenin score 2 x200 c) PCa E-cadherin score 1 d) BPH E-cadherin score 3

BPH and PCa samples were compared in terms of E cadherin and β catenin expression using the SPSS 20.0 program. Variables were expressed in frequency, percentage, mean (Arithmetic mean, median), standard deviation (min-max) with tables and graphs. Independent sample t test and Anova test were used when comparing variables related to patients. Statistical value of $p < 0.05$ was considered significant. In patients with PCa, the correlation of E cadherin and β catenin expression with specified parameters (PSA level < 10 vs. ≥ 10 , Gleason score ≥ 7 vs. < 7 , lymphovascular invasion present/absent, perineural invasion present/absent) was investigated. This study was approved by the Institutional Ethics Committee for the Tekirdağ Namık Kemal University in Research. (Protocol# 2020.34.02.08).

Results

Mean age was 68.3 years and 68.6 years in the 30 patients with BPH who underwent TUR-P and the 59 PCa patients who underwent radical prostatectomy, respectively ($p > 0.05$). Mean β catenin expression was 2.96 ± 0.18 in the 30 patients diagnosed with BPH and 1.80 ± 0.68 in patients with PCa, with a statistically significant difference between these values ($p < 0.001$). Mean E cadherin expression was 3.00 in patients with BPH and 2.38 ± 0.5 in patients with PCa, again with a statistically significant difference between these two groups ($p < 0.001$).

PSA level was < 10 in 33 patients and ≥ 10 in 25 patients with PCa. PSA level was missing/unknown in one patient. Histopathological investigation revealed that Gleason score was < 7 in 39 of the tumors and ≥ 7 in 20. Lymphovascular invasion was noted in only 10 cases, without lymphovascular involvement in remaining

patients. Perineural invasion was observed in a total of 36 cases, without lymphovascular involvement in 23 patients.

Mean E cadherin and β catenin staining scores of PCa patients by

Gleason score, PSA level, presence of lymphovascular invasion and presence of perineural invasion are presented in Table 1 with associated with p values. Mean E cadherin and β catenin expression scores and p values by Gleason scores is presented Table 2.

Table 1. Mean E cadherin and β catenin expression scores and p values by Gleason score, PSA level, lymphovascular invasion and perineural invasion

	Gleason score <7	Gleason score $\geq$ 7	p-value
β-catenin	1.87 $\pm$ 0.73	1.66 $\pm$ 0.57	0.240
E-cadherin	2.43 $\pm$ 0.55	2.28 $\pm$ 0.56	0.320
	PSA <10	PSA $\geq$10	
β -catenin	2.03 $\pm$ 0.68	1.48 $\pm$ 0.58	0.001
E-cadherin	2.42 $\pm$ 0.56	2.36 $\pm$ 0.56	0.667
	No lymphovascular invasion	Lymphovascular invasion	
β -catenin	1.85 $\pm$ 0.69	1.50 $\pm$ 0.70	0.105
E-cadherin	2.40 $\pm$ 0.57	2.40 $\pm$ 0.51	0.905
	No perineural invasion	Perineural invasion	
β-catenin	2.00 $\pm$ 0.70	1.67 $\pm$ 0.66	0.105
E-cadherin	2.47 $\pm$ 0.60	2.35 $\pm$ 0.53	0.356

Table 2. Mean E cadherin and β catenin expression scores and p values by Gleason scores

	Gleason score <7	Gleason score 4+3	Gleason score 4+4
β-catenin	1.90 $\pm$ 0.56	1.86 $\pm$ 0.71	1.45 $\pm$ 0.68
E-cadherin	2.40 $\pm$ 0.51	2.43 $\pm$ 0.55	2.27 $\pm$ 0.64
Gleason score <7			
β -catenin:		p=0.929	p=0.132
E-cadherin:		p=0.848	p=0.756
Gleason score 4+3			
β -catenin	p=0.929		p=0.062
E-cadherin	p=0.848		p=0.469
Gleason score 4+4			
β-catenin	p=0.132	1.67 $\pm$ 0.66	
E-cadherin	p=0.756	2.35 $\pm$ 0.53	

Discussion

E cadherin and β catenin are known to contribute to the pathogenesis of various malignant tumors, including prostate, breast, colorectal and gastric cancers. Developing selective inhibitors for E cadherin may provide useful therapeutic agents for malignant tumors such as prostatic adenocarcinoma [6].

While several studies have evaluated E cadherin and β catenin expression in benign prostate tissue, BPH, prostatic intraepithelial neoplasia (PIN) and PCa, there is limited data on the correlation

between the expression of these molecules in BPH and PCa tissues.

Loss or reduction of E cadherin expression is observed in several poorly differentiated and invasive tumors, reflecting increased tumor progression and metastasis with reduced E cadherin mediated cell to cell contact. Furthermore, abnormal E cadherin expression has been shown to increase the transcriptional activity and dysregulate the cytoplasmic pool of β catenin [19,20].

Olson et al. investigated the effects of E cadherin loss on prostate epithelium using newly developed genetically engineered mouse

models, showing that PIN was triggered by decreased E cadherin in prostatic luminal epithelial cells. Reduced E cadherin was noted in PIN lesions, with increased cytoplasmic and nuclear β catenin in atypical cells. Using various experimental approaches, they showed increased cytoplasmic and nuclear β catenin expression in the event of reduced E cadherin expression, demonstrating increased androgen induced transcription and cell growth. These data show that loss of E cadherin in prostatic epithelial cells is not only associated with oncogenic transformation and expansion but also with epithelial disorganization in addition to inducing cell apoptosis and tissue damage [7].

On the other hand, loss of expression has been demonstrated for e-, n- and p cadherin and α - and β catenin in BPH compared to normal prostate tissue [21].

In the present study, all BPH tissues were hyperplastic, with normal E cadherin and β catenin expression (score 3+). However, there was a statistically significant reduction in E cadherin and β catenin expression in PCa cases. Similar to our study, decreased E cadherin and β catenin expression has been shown in PCa development in the literature [6,8]. Studies in dogs revealed β catenin membranous staining in all normal prostatic epithelial cells while loss of membranous β catenin expression was observed in atrophic prostate epithelium and PCa tissues [22].

In a study conducted with 27 BPH and 45 PCa cases, Musalam et al. showed homogeneous strong and widespread E cadherin expression in normal prostate epithelium, BPH and most PCa cases. There was only a small number of PCa cases with reduced E cadherin expression [6].

In a study investigating the association between PCa and E cadherin, Köksal et al. evaluated E cadherin expression using an immunohistochemical method in 58 radical prostatectomy samples. They found reduced E cadherin staining in cases with a pathological stage of PT2 and pT3a [23].

In our study, there was no statistically significant difference between cases with Gleason score ≥ 7 vs. those with Gleason score < 7 in terms of E cadherin and β catenin expression scores.

In PCa, curative treatment may be given in the low risk group (PSA < 10 , Gleason score < 7 , T2a) based on the European Association of Urology (EAU) risk classification. However, such patients may also be observed with active monitoring without any treatment [2]. The fact that low risk patients may be followed without curative treatment renders this group clinically important [24]. In the present study, a significant decrease was observed in β catenin expression in the low risk group (PSA > 10 , Gleason score > 7 , $> T2a$) compared to the moderate- and high risk groups based on EAU risk classification ($p=0.001$).

Loss of E cadherin and β catenin is known to increase oncogenic transformation, tumor invasiveness and metastatic capacity. However, there was no significant difference between the groups with and without lymphovascular invasion or perineural invasion in terms of E cadherin and β catenin expression.

Limitations

In this study, an equal number of cases was planned to be enrolled in PCa and BPH groups. However, as expected, E cadherin and β catenin expression was found to be 3+ in nearly the entire BPH group. Therefore, cases were mostly selected from PCa cases in order to predict the risk classification (differences between PSA levels, Gleason scores etc.) in the PCa group.

Immunohistochemical E cadherin and β catenin staining was planned to be evaluated in a single blind manner regardless of benign or malignant nature and risk classification. However, since immunohistochemical staining slides were from the same source as the pathology slides, it proved to be impossible for the assessor pathologist to not predict the malignant or benign classification and Gleason scores. For this reason, all slides were evaluated concurrently by two pathologists and immunohistochemical staining was scored based on their consensus in order to avoid bias.

Conclusion

Loss of E cadherin and β catenin is known to contribute to the pathogenesis of prostate carcinoma. The association between carcinogenesis and the expression of these molecules needs to be further elucidated through future molecular studies on this subject. We believe such studies may guide the development of therapeutic targets that are specific for E cadherin and β catenin in the treatment of prostate carcinoma.

Conflict of interests

The authors declare that they have no conflict of interest.

Financial Disclosure

The financial support for this study was provided by the investigators themselves.

Ethical approval

This study was approved by the Institutional Ethics Committee for the Tekirdağ Namık Kemal University in Research. (Protocol# 2020.34.02.08).

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