β-catenin expression in endometrioid type endometrial cancer: Expression patterns and impact on disease outcomes

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Received May 15, 2024; Accepted September 11, 2024

DOI: 10.3892/ol.2024.14713

Abstract. Determination of nuclear and/or cytoplasmic expression of β-catenin by immunohistochemistry in patients with endometrial cancer (EC) may constitute a potential diagnostic method for identifying patients with a catenin β1 (CTNNB1) gene mutation and those at risk of disease recurrence. The present study aimed to investigate β-catenin expression patterns in hysterectomy specimens of patients with endometrioid type EC using immunohistochemistry, and to examine the prognostic impact of β-catenin. The study was a single-institutional, retrospective cohort trial enrolling consecutive patients with a postoperative histopathological diagnosis of endometrioid EC who underwent hysterectomy between January 2015 and December 2018. Histopathology slides from 75 patients were stained with a monoclonal antibody targeting the β-catenin protein. Any percentage of nuclear staining, whether focal or diffuse, was considered 'β-catenin nuclear-positive'. The cytoplasmic staining reaction of β-catenin was assessed based on the percentage of stained cells and staining intensity. Immune-reactivity score (IRS) values were determined by multiplying the scores for the percentage of staining and staining intensity. IRS values 0 to 2 were regarded as negative expression, 3 to 4 as low expression, 6 to 8 as moderate expression, and 9 to 12 as high expression. Recurrence-free survival (RFS) was used as the prognostic endpoint. Only 2 out of 75 tissue samples (2.7%) exhibited nuclear β-catenin expression, with a low staining percentage of 5%. By contrast, cytoplasmic staining was observed in all samples (100%). According to the IRS findings, 1.3% of the samples exhibited negative cytoplasmic expression, 42.7% low expression, 38.7% moderate expression and 17.3% high expression. Cox regression analysis revealed that staining with β-catenin, either nuclear or cytoplasmic, had no impact on RFS, and stage was the sole independent prognostic factor. In conclusion, based on these results, β-catenin expression in endometrioid EC was revealed to be mostly cytoplasmic, with only 2.7% of tissue samples exhibiting nuclear expression. Overall, β-catenin expression has no impact on RFS.

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Key words: endometrioid endometrial cancer, β-catenin, prognosis, survival

Introduction

The most recent data from 2020 indicates that endometrial cancer (EC) is the most common gynecologic malignancy in Europe and the second most common worldwide (1). Endometrioid type EC accounts for ~80% of all cases of EC. In the absence of recurrence, the 5-year overall survival (OS) rate for endometrioid EC is 91.9%. However, recurrence occurs in ~8% of patients, and OS decreases to 77% for vaginal recurrence alone and 36% for recurrence elsewhere (2). It is therefore essential to identify cases at risk of recurrence in order to optimize the disease management. Traditional risk factors for recurrence in EC include stage, histological type, grade, age and lymphovascular space involvement (LVSI) (3).

In 2013, The Cancer Genome Atlas (TCGA) project identified four distinct molecular subgroups of EC associated with prognosis (4). These subgroups consisted of EC with a high mutation rate in the polymerase-ε (POLE) exonuclease domain (POLE-mutated), microsatellite-instability-high (MSI-H) EC, EC with low mutation rate and low somatic copy number alteration [non-specific molecular pattern (NSMP)], and EC with low mutation rate but high somatic copy number alteration rates and TP53 mutations.

A recent systematic review and meta-analysis has reported that endometrioid EC cases with catenin β1 (CTNNB1) exon 3 mutations are associated with higher recurrence rates and poorer disease outcomes (5). The CTNNB1 gene encodes the protein β-catenin. In normal epithelial cells, β-catenin is localized in the cell membrane together with the E-cadherin protein. Both are involved in adhesions between the cells through adherens junctions. Mutations in exon 3 of the CTNNB1 gene or activation of the canonical Wnt pathway cause translocation of β-catenin from the cell membrane to

the cytoplasm and nucleus, where it accumulates (6). This leads to the loss of cell-cell adhesions and is hypothesized to contribute to uncontrolled cell proliferation and metastasis (6). It has been suggested that cases with CTNNB1 mutation may represent a fifth molecular subgroup of EC (7).

The new International Federation of Gynecology and Obstetrics (FIGO) 2023 staging system for EC promotes the use of both molecular classification and traditional prognostic factors (8). However, genome sequencing is an expensive and complex procedure, which represents a significant barrier to the widespread use of molecular classification in clinical practice. Determination of nuclear and/or cytoplasmic expression of β-catenin by immunohistochemistry may constitute a potential diagnostic method for identifying patients with a CTNNB1 mutation and those at risk of disease recurrence.

The current study primarily aimed to investigate β-catenin expression patterns in hysterectomy specimens from patients with endometrioid type EC using immunohistochemistry. Secondly, the study examined the prognostic impact of β-catenin expression on recurrence-free survival (RFS).

Materials and methods

Study design and patients. The present study was a single-institutional (Antalya Training and Research Hospital, Antalya, Turkey), retrospective cohort trial enrolling consecutive patients with a postoperative histopathological diagnosis of endometrioid type EC who underwent primary surgery, including at least total hysterectomy plus bilateral salpingo-oophorectomy (TH/BSO) between January 2015 and December 2018. The exclusion criteria were as follows: i) Non-endometrioid and mixed type histotypes; ii) receipt of neoadjuvant chemotherapy, radiotherapy or hormone therapy; and iii) the presence of synchronous malignancy. The study was approved by the Ethics Committee of the Antalya Training and Research Hospital (approval no. 21/14; Antalya, Turkey) and it conformed to the provisions of The Declaration of Helsinki. Due to the retrospective nature of the study, the need for informed consent was waived by the ethics committee.

Clinicopathological data, including age, surgical procedures, tumor size, grade, LVSI, myometrial invasion, cervical involvement, adnexal involvement, lymph node involvement, number of lymph nodes removed, the stage of the disease, adjuvant therapy, length of follow-up, disease recurrence and survival status on the date of the most recent follow-up were retrieved from the patient charts and institutional electronic database following the receipt of ethics approval.

A total of 121 patients with EC were treated at Antalya Training and Research Hospital during the study period, of whom 85 were diagnosed with pure endometrioid EC. Subsequently, 10 patients were excluded from the study, 4 due to neoadjuvant chemotherapy, 1 due to neoadjuvant radiotherapy and 5 due to synchronous malignancy. The final analyses thus involved 75 patients who met the eligibility criteria.

Immunohistochemical studies. Tissue samples from 75 patients were extracted from the archives of the Department of Pathology. The samples had been fixed with 10% neutral buffered formalin solution for 24 h at room temperature and embedded in paraffin. Tissue blocks that best reflected the lesion and the area most suitable for immunohistochemical staining were selected. Tissue sections (3 μm) were cut from tissue blocks onto pre-coated slides. The samples were subsequently incubated at 60˚C for 60 min, deparaffinised by passage through xylene (three times for 5 min each) and rehydrated by successive immersion in 100, 96, 90, 80 and 70% alcohol for 5 min each. Immunohistochemistry was performed in an automated platform (Shandon; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions using the monoclonal mouse antibody targeting β-catenin-1 protein (catalog no. IR702; 1:100; Dako; Agilent Technologies, Inc.) at room temperature for 2 h. The blocking reagent (catalog no. TA-125-UB; concentrated polymer-based protein-free blocking agent; Thermo Fisher Scientific, Inc.) was used for 10 min at room temperature. A ready-to-use secondary antibody (catalog no. TL-125-HL; enzyme-labelled polymer; Thermo Fisher Scientific, Inc.) was applied 30 min at room temperature. The sections were then stained using diaminobenzidine as a chromogen and hematoxylin for counterstaining. Both staining steps were performed for 5 min at room temperature.

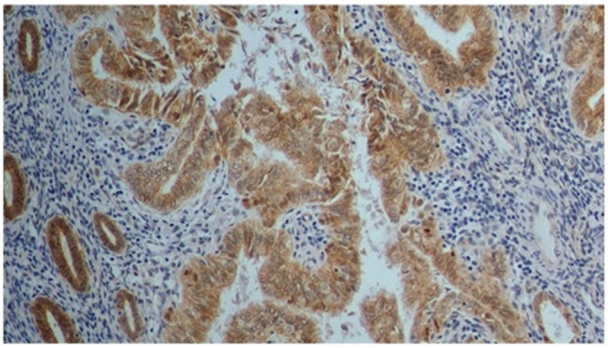

Figure 1. Nuclear β-catenin expression (magnification, x200).

The stained tissue slides were assessed in terms of staining reaction (brown granules) under a light microscope at x40 magnification by a gynecological pathologist (HTY) blinded to the clinicopathological data. Cytoplasmic staining in cells lining normal endometrial glands adjacent to the tumor tissue was used as a positive control for β-catenin staining intensity. Any percentage of nuclear staining, whether focal or diffuse, was considered 'β-catenin nuclear positive' (Fig. 1) (9).

The cytoplasmic staining reaction of β-catenin (tumor cells with cytoplasm containing brown granules) was assessed and scored based on the percentage of stained cells and staining intensity (10). The percentage of staining with β-catenin was calculated as the number of immunopositive cells divided by the total number of cells counted. The grading criteria for the percentage of staining were as follows: 0, no staining; 1, ≤10%; 2, 11-50%; 3, 51-80%; and 4, ≥81%. The staining intensity was graded semi-quantitatively based on the shade of brown granules as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining (Fig. 2). The immune-reactivity score (IRS) was determined by multiplying the percentage of staining and staining intensity scores. IRS values 0 to 2 were regarded as negative expression, 3 to 4 as low expression, 6 to 8 as moderate expression, and 9 to 12 as high expression.

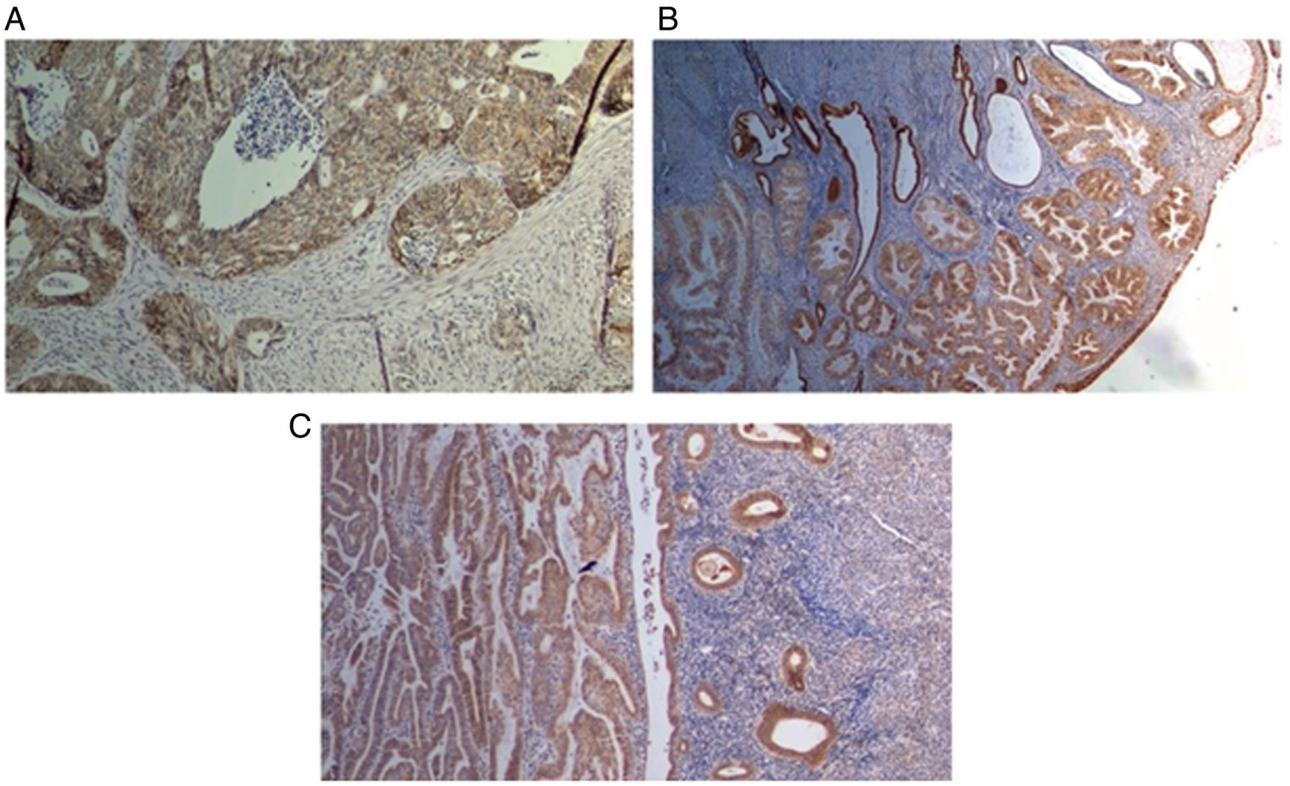

Figure 2. Cytoplasmic β-catenin expression (magnification, x200): (A) Weak staining, (B) moderate staining and (C) strong staining.

Statistical analysis. Statistical analyses were performed using SPSS Statistics version 20.0 (IBM Corp.) software. Binary variables were reported as counts and percentages, and continuous variables as median and range. RFS was used as the prognostic endpoint. Cox proportional hazards regression models were used to determine the prognostic roles of β-catenin and various clinicopathologic factors on RFS. The model results were presented as hazard ratios (HRs) with 95% confidence intervals (CIs). $P<0.05$ was considered to indicate a statistically significant difference; therefore, variables with a $P<0.05$ in univariate analyses were included in the multivariate analyses. RFS was defined as the duration in months between the date of surgery and the date of first recurrence or mortality from any cause, whichever occurred first, or the date of most recent visit for patients alive without disease.

Results

Table I presents the clinicopathological characteristics of the patients. The median age at surgery was 57 years. Almost all patients (94.7%) underwent lymph node dissection in addition to TH/BSO, with only 4 patients undergoing TH/BSO alone. The majority of the patients had FIGO stage I disease (72.0%). The distribution of tumor grades was as follows: Grade 1, 54.7%; grade 2, 29.3%; and grade 3, 16.0%. Deep (≥50%) myometrial invasion was observed in 46.7% of patients, LVSI in 32.0%, cervical invasion in 14.7%, adnexal involvement in 5.3% and lymph node involvement in 18.7%. Approximately half of the patients received no adjuvant therapy, while 12.0% received brachytherapy alone, 18.7% external beam radiotherapy with or without brachytherapy, 1.3% chemotherapy alone and 18.7% chemoradiation. Overall, 3 of the 75 patients were lost to follow-up and 1 died due to pulmonary thromboembolism on the day of surgery. During a median follow-up time of 53 months, 6 of the remaining 71 patients (8.4%) experienced disease recurrence. The median time to recurrence was 21 months. At the time of analysis, 60 patients (83.3%) were alive without disease, 5 (6.9%) were alive with disease, 3 (4.0%) had died from disease and 4 (5.3%) had died from other causes. The estimated 4- and 5-year RFS rates were 80.9 and 74.0%, respectively.

The immunohistochemical staining features of tumor cells with β-catenin are summarized in Table II. Nuclear staining with β-catenin was determined only in 2 of 75 tissue sample (2.7%). Percentage values of nuclear staining were 5% in both samples. On the other hand, cytoplasmic staining with β-catenin was observed in all tissue samples (100%). Percentage values of tumor-cell cytoplasmic staining were between 11 and 50% in 2.7% of tissue samples, between 51 and 80% in 57.3% and ≥81% in 40.0%. The intensity of cytoplasmic staining with β-catenin was weak in 42.7% of the samples, moderate in 40.0%, and strong in 17.3%. According to the IRS analysis, 1.3% of the samples exhibited negative cytoplasmic β-catenin expression; 42.7% low-expression; 38.7%, moderate-expression; and 17.3%, high-expression.

Analyses of factors associated with RFS are presented in Table III. In the univariate analysis, two variables were identified as significantly associated with disease prognosis: Lymph node involvement and FIGO stage. The localization, percentage and intensity of β-catenin expression, and the IRS score were each tested as potential covariates; however, no statistically significant relationship was identified. Thus, these variables

Table I. Clinicopathologic characteristics of patients.

Variables	Values
Median age (range), years	57 (39-77)
Surgical procedure, n (%)	
TH/BSO alone	4 (5.3)
TH/BSO plus systematic lymph node dissection	71 (94.7)
Median no. of lymph nodes removed (range)	36 (9-104)
Median tumor size (range), cm	3 (0.20-15)
FIGO grade, n (%)	
Grade 1	41 (54.7)
Grade 2	22 (29.3)
Grade 3	12 (16.0)
Lymphovascular space involvement, n (%)	24 (32.0)
Deep (≥50%) myometrial invasion, n (%)	35 (46.7)
Cervical involvement, n (%)	11 (14.7)
Adnexal involvement, n (%)	4 (5.3)
Lymph node involvement, n (%)	14 (18.7)
FIGO stage, n (%)	
Stage I	54 (72.0)
IA	37 (49.3)
IB	17 (22.7)
Stage II	6 (8.0)
Stage III	12 (16.0)
IIIA	1 (1.3)
$IIIC_1$	8 (10.7)
$IIIC_2$	3 (4.0)
Stage IVB	3 (4.0)
Adjuvant therapy, n (%)	38 (50.7)
Brachytherapy alone	9 (12.0)
EBRT +/- brachytherapy	14 (18.7)
Chemotherapy alone	1 (1.3)
Chemotherapy plus EBRT	14 (18.7)
Median follow up time (range), months	53 (8-105)
Lost to follow-up, n (%)	3 (4.0)
Recurrence, n (%)	6/71 (8.4)
Median time to recurrence (range), months	21 (13-79)
Survival status, n (%)[a]	
Alive with no evidence of disease	60 (83.3)
Alive with disease	5 (6.9)
Dead of disease	3 (4.2)
Dead of other reasons	4 (5.6)
Recurrence-free survival, median (95% CI)	Not reached
48 months, %	80.9
60 months, %	74.0

[a]Available for 72 out of 75 patients. TH, total hysterectomy; BSO, bilateral salpingo-oophorectomy; FIGO, International Federation of Gynecology and Obstetrics; EBRT, external beam radiotherapy; CI, confidence interval.

Table II. Immunohistochemical staining features of tumor cells with β-catenin.

Variables	No. of patients (%)
Nuclear staining of tumor cells with β-catenin	2 (2.7)
Percentage of tumor-cell staining	
No staining	73 (97.3)
≤10%	2 (2.7)
11-50%	-
51-80%	-
≥81%	-
Cytoplasmic staining of tumor cells with β-catenin	75 (100)
Percentage of tumor-cell staining	
No staining	-
≤10%	-
11-50%	2 (2.7)
51-80%	43 (57.3)
≥81%	30 (40.0)
Staining intensity	
No staining	-
Weak	32 (42.7)
Moderate	30 (40.0)
Strong	13 (17.3)
Immune-reactivity score	
0-2 (Negative expression)	1 (1.3)
3-4 (Low-expression)	32 (42.7)
6-8 (Moderate-expression)	29 (38.7)
9-12 (High-expression)	13 (17.3)

were excluded from the multivariate analysis models. The multivariate analysis revealed that the FIGO stage (P=0.007) was the sole independent significant predictor of RFS. While patients with extrauterine disease (FIGO stage III-IV) had an 8.1-fold (95% CI, 1.484-44.310; P=0.016) higher risk of recurrence or death compared with those with disease confined to the uterus (stage I-II), patients with stage II-IV disease had a 14.6-fold (95% CI, 1.688-26.332; P=0.015) higher risk of recurrence or death compared with those with stage I disease.

Subsequently, two additional regression analyses were performed to explore the prognostic effect of variables in uterine-confined (stages I-II) and extrauterine diseases (stage III-IV) (Table IV). However, no independent prognostic factor was identified in either group.

Discussion

The present study primarily investigated β-catenin expression patterns in endometrioid type EC using immunohistochemistry. It also examined the prognostic impact of β-catenin expression on the RFS of patients. The study revealed that the expression of β-catenin in endometrioid EC was mostly cytoplasmic, with only 2.7% of tissue samples exhibiting nuclear expression. The study findings also showed that β-catenin expression, either nuclear or cytoplasmic, had no impact on

Table III. Cox regression analyses for factors associated with recurrence-free survival.

	Univariate			Multivariate		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	0.975	0.881-1.079	0.626	-	-	-
Tumor size, cm	1.254	0.880-1.787	0.211	-	-	-
FIGO grade						
Grade 1	1	-	0.245	-	-	-
Grade 2	2.962	0.268-32.710	0.376	-	-	-
Grade 3	7.018	0.684-71.955	0.245	-	-	-
Lymphovascular space involvement	3.310	0.593-18.469	0.172	-	-	-
Deep (≥50%) myometrial invasion	1.889	0.345-10.356	0.464	-	-	-
Lymph node involvement	8.529	1.560-46.628	0.013[a]	-	-	0.405
FIGO stage	4.840	1.667-14.052	0.004[a]	7.012	1.718-28.627	0.007[a]
Stage I vs. II-IV	14.602	1.688-26.332	0.015[a]			
Stage I-II vs. III-IV	8.109	1.484-44.310	0.016[a]			
Adjuvant therapy	3.243	0.373-28.190	0.286	-	-	-
Number of lymph nodes removed	1.014	0.973-1.056	0.519	-	-	-
Nuclear staining with β-catenin	0.048	0.006-1.996	0.814	-	-	-
Cytoplasmic staining with β-catenin						
Percentage of staining	1.037	0.937-1.147	0.482	-	-	-
Intensity of staining	0.332	0.077-1.443	0.141	-	-	-
Immune-reactivity score	0.758	0.498-1.153	0.196	-	-	-

[a]P<0.05. HR, hazard ratio; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics.

Table IV. Unadjusted Cox regression analyses for factors associated with recurrence-free survival in stage I-II and stage III-IV diseases.

	Stage I-II			Stage III-IV		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	0.900	0.718-1.128	0.359	1.007	0.912-1.113	0.885
Tumor size, cm	2.430	0.116-51.017	0.568	0.657	0.302-1.429	0.289
FIGO grade	2.862	0.354-23.144	0.324	1.552	0.398-6.051	0.526
Lymphovascular space involvement	0.507	0.058-4.461	0.542	1.964	0.541-7.138	0.660
Deep (≥50%) myometrial invasion	1.155	0.072-18.594	0.919	0.779	0.080-7.566	0.830
Number of lymph nodes removed	1.005	0.953-1.059	0.863	1.009	0.968-1.051	0.680
Nuclear staining with β-catenin	0.048	0.005-2.111	0.909	0.044	0.008-1.611	0.850
Cytoplasmic staining with β-catenin						
Percentage of staining	0.970	0.819-1.150	0.729	1.075	0.929-1.245	0.332
Intensity of staining	0.492	0.057-4.219	0.518	0.636	0.100-4.020	0.630
Immune-reactivity score	0.744	0.328-1.688	0.479	0.947	0.614-1.461	0.807

HR, hazard ratio; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics.

disease recurrence or death, while the only independent factor affecting prognosis was the stage of the disease.

There is growing evidence in the literature suggesting that endometrioid EC cases with CTNNB1 exon 3 mutations exhibit worse oncological outcomes compared with those without CTNNB1 mutations (5). A combined analysis of the Post Oper ative Radiation Therapy in Endometrial Carcinoma cohorts including patients with high-intermediate-risk EC revealed an independent intermediate prognostic value for CTNNB1 mutations in the group of patients with NSMP (11). Kurnit *et al* (12) investigated the impact of somatic mutations on disease outcomes in 245 patients with endometrioid EC,

53 of whom had a CTNNB1 mutation. The authors reported that CTNNB1 mutated tumors were significantly associated with grade 1-2 tumors and with lower rates of deep myometrial invasion and LVSI, while CTNNB1 mutation, age and TP53 mutation were identified as independent predictors of poor RFS. In a case-control study involving patients with stage I, grade 1 endometrioid EC (n=311), Moroney *et al* (13) compared recurrent cases (n=18) with non-recurrent matched controls (n=29). The authors observed that the frequency of both CTNNB1 mutations and MSI-H was significantly higher in the recurrent cases compared with in the controls (CTNNB1, 60 vs. 28%, respectively). A recent meta-analysis (5) examining the prognostic value of CTNNB1 mutations in early-stage (stage I-II) endometrioid EC also revealed a significant association between CTNNB1 mutations and the absolute number of recurrences. CTNNB1 mutations were detected in 52% of recurrent cases versus 25% of non-recurrent cases. Although the authors initially failed to demonstrate an association between CTNNB1 mutation and worse disease-free survival (DFS), the association became significant following the exclusion of patients with a known molecular status other than NSMP. However, it should be noted that this meta-analysis included only four studies in the assessment of absolute number of recurrences and only three in the DFS analysis (5). Further studies are therefore needed to clarify the relationship between CTNNB1 mutation and disease outcomes.

According to TCGA data (4), CTNNB1 exon 3 mutations are detected in ~30% of all endometrioid EC cases, and particularly in approximately half of endometrioid EC cases with NSMP. The detection of CTNNB1-mutated cases with a cost-effective and easily accessible diagnostic method may provide appropriate guidance for adjuvant treatment and post-treatment follow-up of patients with low-risk endometrioid EC (5). Previous studies have suggested that determining the accumulation of β-catenin within the nucleus by means of immunohistochemistry is a useful method for predicting possible mutations in the CTNNB1 gene (7,9,14,15). Kim *et al* (9) examined the immunohistochemical localization of β-catenin in 53 CTNNB1-mutated and 46 non-mutated patients with EC. The authors reported that nuclear β-catenin expression was identified in 45 out of 53 patients, with 100% specificity in distinguishing CTNNB1-mutated patients from CTNNB1 wild-types, although the sensitivity was lower (84.9%). Moreover, the percentage of tumor-cell nuclear staining with β-catenin was only 5-10% in nearly half the CTNNB1-mutated cases. Costigan *et al* (14) investigated the feasibility of β-catenin immunohistochemistry for identifying endometrioid EC cases harboring CTNNB1 mutations in a cohort consisting of 39 CTNNB1-mutated patients and 40 CTNNB1 wild-type patients. The authors reported that nuclear β-catenin expression was significantly associated with the presence of a CTNNB1 exon 3 mutation, with a sensitivity of 91%, specificity of 89%, positive predictive value of 89% and negative predictive value of 84%. The authors also noted that nuclear β-catenin expression in CTNNB1-mutated tumors tended to be focal (75% of tumors exhibit staining in <50% of tumor nuclei), although a strong staining intensity was present in almost all cases. Ruz-Caracuel *et al* (15) assessed the correlation between CTNNB1 mutations and nuclear β-catenin expression in a large cohort of 218 early-stage low-grade endometrioid EC cases. The specificity and sensitivity of nuclear β-catenin expression in identifying CTNNB1 mutation were reported at 93 and 53%, respectively. Finally, a meta-analysis of 15 studies involving >1,000 patients revealed that nuclear β-catenin expression may be an accurate surrogate for the CTNNB1 exon 3 mutation in EC, with a pooled sensitivity of 0.88 (95% CI, 0.81-0.93), a specificity of 0.85 (95% CI, 0.81-0.88) and a high overall diagnostic accuracy (area under the curve=0.91) (7).

In light of TCGA data reporting that ~30% of endometrioid ECs carry CTNNB1 mutations (4), and data from a recent meta-analysis (7) showing >85% sensitivity and specificity for β-catenin immunohistochemistry, nuclear expression of β-catenin may be expected in ~25% of endometrioid type EC tissue samples. In a study by Kim *et al* (9), which included all histotypes of EC, the authors revealed that 63 out of 345 patients (18%) had a CTNNB1 exon 3 mutation. Among the CTNNB1-mutated EC cases, 84.9% exhibited nuclear β-catenin expression, corresponding to ~15% of all EC cases. In the present study, nuclear β-catenin expression was detected in only 2 (2.7%) out of 75 endometrioid EC tissue samples, a much lower figure than expected. Similarly to the present study, Ruz-Caracuel *et al* (15) identified a CTNNB1 mutation in only 8.7% of patients with endometrioid EC, of whom slightly more than half (~5%) had nuclear β-catenin expression. The variations in nuclear β-catenin expression rates between studies may be due to differences in the antibodies and procedures used during immunohistochemical examinations. Moreover, there are no standardized diagnostic criteria for the assessment of β-catenin immunohistochemistry in terms of the percentage of tumor cells stained, staining intensity and, ultimately, the degree of expression. Additionally, potential bias in the selection of patients may have contributed to the differences observed.

CTNNB1 exon 3 mutations are classically regarded as causing reduced degradation of β-catenin protein, followed by its accumulation in the cytoplasm and subsequent translocation to the nucleus (6). Kim *et al* (9) reported a 100% rate of cytoplasmic β-catenin expression in 46 randomly selected CTNNB1 wild-type EC samples, compared with 8/53 (15%) in CTNNB1-mutated cases. It has been shown that Wnt pathway activation can occur independently of nuclear β-catenin accumulation in CTNNB1 wild-type cases (16). In the present study, in contrast to nuclear expression, cytoplasmic staining with β-catenin was observed in all tissue samples (100%), with 42.7% exhibiting low expression, 38.7% moderate and 17.3% high. These results support that the actual number of CTNNB1 exon 3-mutated cases in the present study was low in parallel with the number of nuclear β-catenin-positive cases. In the present study, nuclear or cytoplasmic β-catenin expression had no effect on RFS, and stage emerged as the only independent factor associated with prognosis. In contrast to the previous literature, the principal reason for the inability of the present study to show the effect of nuclear β-catenin expression on survival was the very low number of positive patients. On the other hand, and consistent with the current results, Imboden *et al* (17) compared 21 patients with low-risk EC (stage I, grade 1-2) with recurrence and 20 matched controls without recurrence. The authors observed similar CTNNB1 mutation rates between the groups (44.4% in the recurrence group vs. 55.5% in controls). Although cytoplasmic β-catenin

expression has been reported to be associated with poor disease outcomes in patients with renal cell carcinoma (18), no previous studies in the literature have focused on the relationship between cytoplasmic β-catenin expression and disease recurrence in EC. From that perspective, the present study represented an important addition to the existing literature.

The Ki-67 marker of cellular proliferation is increasingly being utilized as a primary outcome measure in EC research. A positive association has been identified between high Ki-67 levels and several clinicopathological parameters, including tumor grade, depth of myometrial invasion, stage of the disease and cancer-specific survival (19). However, there are currently no established guidelines for a standardized assessment and scoring of this marker in EC. Furthermore, the relationship between Ki-67 and the β-catenin signaling pathway remains to be elucidated. Therefore, the Ki-67 proliferation index was not utilized in the methodology of the present study. Further research is required to elucidate the relationship between distinct protein expressions and survival in EC, whether in combination or as individual factors.

As with all studies, the analyses presented in the present study are not without limitations. The study was retrospective in design, conducted at a single tertiary-care center, and included a relatively small number of cases with a single histological type. This resulted in a lack of comparative data with a second cohort comprising of cases of different histotypes and stages, thereby limiting the generalizability of the findings. The potential subjectivity in the interpretation of immunohistochemical results could not be excluded due to the single-center nature of the current study and the lack of external validation. In order to test the validity of the results, the present study performed a comprehensive search of the literature for the online availability of databases regarding β-catenin expression in EC. However, despite our best efforts, we were unable to identify an online database. Further comparative studies, particularly those utilizing new methods such as machine learning for the interpretation of immunohistochemical staining, may test the validity of the findings of the present study and reduce the interference of subjective factors. In addition, the present study investigated the prognostic role of β-catenin expression solely by means of immunohistochemistry, and the results were not confirmed by molecular testing. Furthermore, the lack of utilization of TCGA molecular classification precludes the drawing of definitive conclusions regarding the significance of β-catenin expression in different molecular groups.

In conclusion, based on the results of the present study, β-catenin expression in endometrioid EC was mostly cytoplasmic, with only 2.7% of tissue samples exhibiting nuclear expression. Furthermore, β-catenin expression had no impact on disease recurrence and death.

Acknowledgements

Not applicable.

Funding

This study was performed with the support of Education Planning Committee of the Antalya Training and Research Hospital, Antalya, Turkey (grant no. 2022/335).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

OH conceptualized the study, collected, validated and analyzed the data, wrote the original draft and acquired funding. AA designed the methodology, validated and analyzed the data, wrote the original draft, and reviewed and edited the manuscript. HTY conceptualized the study, designed the methodology and collected the data. MuG, NY and SK collected and interpreted the data. MeG contributed to the analysis and interpretation of the data. IU was the project administrator, contributed to the design of the study and critically reviewed the intellectual content. TT conceptualized the study, designed the methodology, validated and analyzed the data, and reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. AA and OH confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Antalya Training and Research Hospital (approval no. 21/14). Although the Ethics Committee waived the requirement for informed consent due to the retrospective nature of the study, written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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