Original Article

β-Catenin and FAT1 Protein Expressions for Prediction of Survival Outcome in Type I Endometrial Cancer

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Background: Endometrial cancer (EC) is the second most common female genital tract malignancy and has adverse outcome in the advanced stage. A prognostic marker is needed for marking an accurate prognostic.

Objective: To evaluate expression and clinical outcome of CTNNB1 (β-catenin) and FAT atypical cadherin 1 (FAT1), by using immunohistochemical staining in EC type I.

Materials and Methods: Seventy-two EC type I cases were selected from Songklanagarind Hospital with clinical data collection. All cases were evaluated by immunohistochemistry using antibodies against β-catenin and FAT1.

Results: All cases of EC type I were β-catenin positive and FAT1 negative. Moderate and strong intensity (2+ and 3+) β-catenin cytoplasmic staining showed statistically significant association with low grade EC, and low risk of recurrence disease or metastasis ($p<0.05$). β-catenin nuclear staining was present in 19 of 28 cases (68%) of EC grade 1 and associated with low grade EC. β-catenin and FAT1 expression were not associated with 5-year overall survival in both univariate and multivariate analyses.

Conclusion: β-catenin cytoplasmic staining may be associated with low grade EC, and helpful to predict recurrent risk. However, FAT1 and β-catenin expressions have no statically significant correlation with 5-year overall survival and cannot be used to determine the prognosis in type I EC patients.

Keywords: β-catenin, FAT1, Immunohistochemistry, Metastasis, Recurrence, Survival outcome, Type I endometrial cancer

Received 21 September 2020 | Revised 16 February 2021 | Accepted 22 February 2021

J Med Assoc Thai 2021;104(5):757-63

Website: http://www.jmatonline.com

Endometrial cancer (EC) is the second most common malignancy of the female genital tract, and the incidence is increasing worldwide[1-4]. Patients with EC, in advanced stages, usually have poor survival rates[5]. Hence, a new prognostic marker is needed for making an accurate, prognostic.

EC is divided into two major types, type I and type II[6-8]. Type I cancer, endometrioid carcinoma, is the most common type, accounting for approximately 80% of all cases. Pathogeneses of this tumor include mutations of phosphatase and tensin homologue (PTEN) and CTNNB1 (β-catenin) gene mutation. Type II cancer includes non-endometrioid cancer, such as serous and clear cell carcinoma[9,10]. Treatment of type I and type II EC are different, and type II cancer requires a more aggressive therapy[11-13].

β-catenin is a transcriptional factor, which is degraded in adenomatous polyposis coli (APC)/β-catenin complex. Changing of these signaling pathways causes enhanced proliferation during EC carcinogenesis[14,15]. Many studies have suggested that β-Catenin expression in the nucleus is associated with the prognosis of EC patients[16-19].

FAT atypical cadherin 1 (FAT1) is a member of FAT cadherin and locates on chromosome 4q34 to 35. The functions of FAT1 are to inhibit cell proliferation by binding to β-catenin transcriptional factor[20-28]. FAT1 mutation may be associated with type I EC, because of inactivated FAT1 proteins increasing β-catenin protein in the cytoplasm and nucleus that enhances cell proliferation[29].

There are limited studies concerning FAT1 expression in cancer[30-32]. Reduction of FAT1 in the cell membranes, of cancer cells, and increased β-catenin expression might be associated with a poor clinical outcome. However, the association between

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How to cite this article:

Kanjanapradit K, Wetwittayakhlung P, Rattanaburi A. β-Catenin and FAT1 Protein Expressions for Prediction of Survival Outcome in Type I Endometrial Cancer. J Med Assoc Thai 2021;104:757-63.

doi.org/10.35755/jmedassocthai.2021.05.12037

FAT1 or β-catenin expression and prognosis of EC is still unclear. Therefore, the objective of the present study was to evaluate expression and prognostic values of FAT1 and β-catenin in type I EC.

Materials and Methods

Study population

Formalin-fixed paraffin embedded tissue samples, and tissue were selected, retrospectively, from Songkhlanagarind Hospital between January 2007 and December 2011. Clinical information, including type of cancer, stage, and treatment were collected. The data of five years survival and recurrence rate were collected from date of diagnosis to the last follow-up date. The latest follow-up time was in 2016.

The study groups consisted of 72 cases of Type I EC. All cases had paraffin block and complete clinical data. All specimens were reviewed by one pathologist and the cases with insufficient tissue for immunostaining were excluded. Patients who received neoadjuvant chemotherapy, radiotherapy, and prior cancer treatment were excluded. The present study received ethical approval by Ethical Committee of Faculty of Medicine, Prince of Songkla University (REC 59-366-05-1) before performing the research.

Immunohistochemistry

Tissue samples were fixed in 10% neutral buffer formalin and embedded in paraffin. Immunohistochemical staining was performed on the selected slides, using rabbit polyclonal FAT1 antibody (dilution 1:50, Sigma) and mouse monoclonal β-catenin antibody (dilution 1:100, MARQUE) in all cases. Staining was performed with the Leica BOND-MAX automated immunostaining.

Interpretation

The results of the immunostaining were evaluated by cellular localization, intensity, and percentage of positive tumor cells. Membranous staining of more than 90% of the tumor cells was considered preserved and staining of 90% or less of tumor cells was considered reduced for expression of FAT1 and β-catenin. Cytoplasmic staining of FAT1 and β-catenin was scored as score 0 (negative) and positive (positive greater than 0%) from 1+ to 3+, based on intensity (Figure 1). The nuclear staining of FAT1 and β-catenin were considered as absent (negative) or present (positive greater than 0%)[32].

Statistical analysis

Statistical analyses used chi-square test and Pearson correlation test to evaluate correlations between all variables. The survival analysis used Kaplan-Meier curves and log-rank test. Prognostic values of all variables were calculated by univariate or multivariate Cox proportional hazard regression models. The statistical significance was defined as p-value less than 0.05. All statistical analyses were calculated by R program studio 3.3.1.

Results

Demographic data

The mean age of patients was 57.2 years, and most cases (66.7%) were menopause. Mean of body mass index (BMI) was 27.5 kg/m^2. Most cases were diagnosed at stage 1 (59.7%) and grade 1 (51.4%). Tumor recurrence or metastasis were found in 22.2% of cases. The maximum follow-up time was 60 months. The 5-year overall survival (OS) rate was 77.8%. Sixteen patients (22.2%) died of disease during the study period.

Immunohistochemistry

The expression of β-catenin immunohistochemical staining is shown in Table 1. Cytoplasmic staining for β-catenin was positive in 13 (18%), 46 (64%), and 13 (18%) cases (intensity from 1+ to 3+, respectively). β-catenin membranous expression was preserved in 46 (64%) cases. β-catenin nuclear expression was present in 28 (39%) cases. Staining for FAT1 was negative in all 72 cases, while all positive control slides that were normal for colonic mucosa, were positive.

Demographic data and associations between β-catenin expressions with clinicopathological factors such as stage, grade, nodal status, and recurrence or metastasis status are shown in Table 2. There was no statistically significant association between β-catenin membranous expressions and clinicopathological factors. β-catenin cytoplasmic expression was associated with low grade cancer (p=0.037), and low recurrence rate or metastasis (p=0.020). β-catenin nuclear staining was present in 68% of grade1 cases. There was correlation between nuclear staining and low-grade tumor (p=0.031).

Survival analysis

The Kaplan-Meier curve of β-catenin membranous expression, cytoplasmic expression and nuclear expression in EC showed no significant effect on 5-year OS, by log rank test (Figure 2-4). Univariate

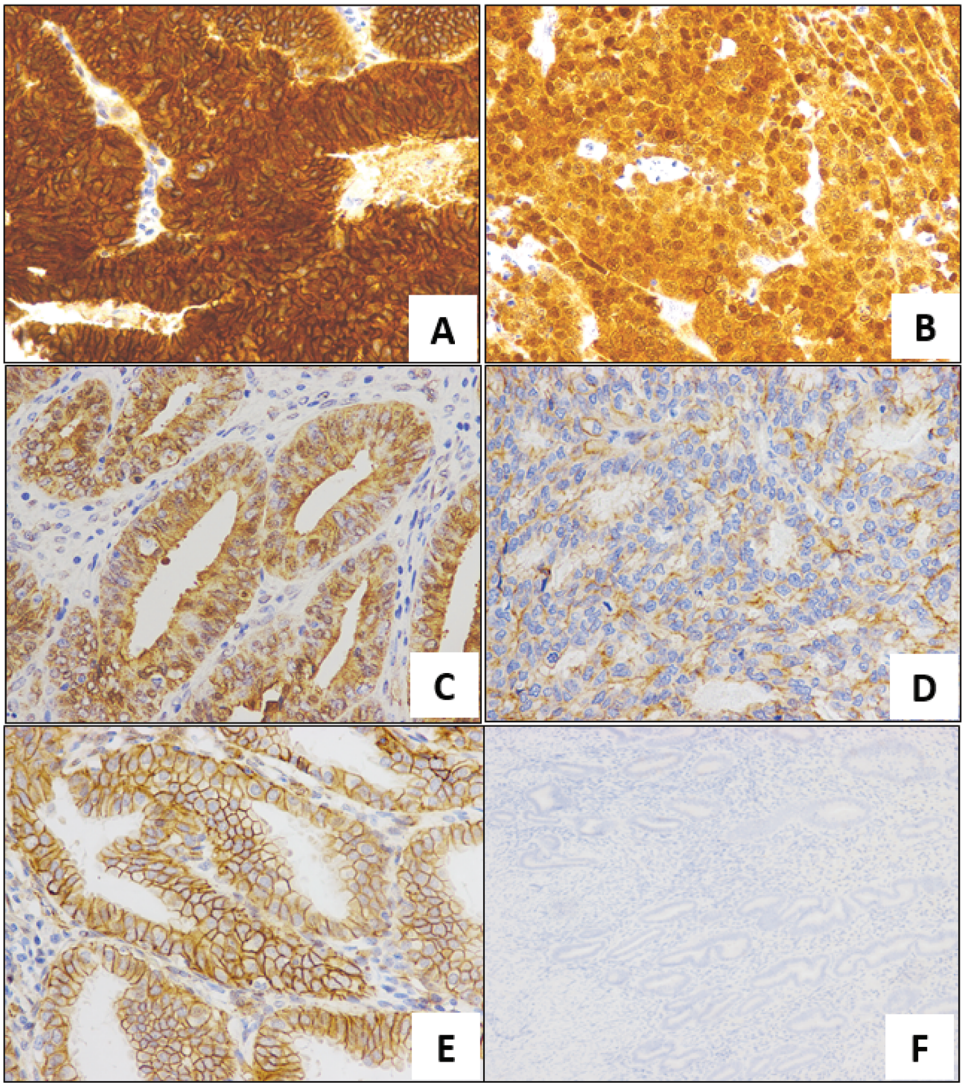

Figure 1. The immunohistochemical staining of β-catenin in cytoplasmic of tumor cells, in three intensity levels: 3+ (A), 2+ (B), 1+ (C), and negative staining (D). The positive immunostaining of β-catenin in cell membranes (E). The negative immunostaining for FAT1 (F).

Table 1. The results of β-catenin immunohistochemical staining

Immunohistochemical staining	Result (n=72); n (%)
β-catenin cytoplasmic staining	
1+	13 (18)
2+	46 (64)
3+	13 (18)
β-catenin membranous staining	
Preserve	46 (64)
Reduce	26 (36)
β-catenin nuclear staining	
Presence	28 (39)
Absence	44 (61)

analysis showed stage, grade, nodal metastasis, and recurrent or metastasis had significant effect on 5-year OS, by cox proportional hazard model. Whereas β-catenin expression did not have any significant effect on 5-year OS. Multivariate analyses revealed that age, stage, grade, nodal metastasis, and recurrent or metastasis were independent prognostic factors for 5-year OS. In age of diagnosis, those of 57 years of age or more had a higher chance of dying than those of younger age. High grade, high stage, positive nodal metastasis, and patients with recurrent or metastasis had poor survival outcomes. Univariate and multivariate cox proportional hazard model analyses results are summarized in the Table 3.

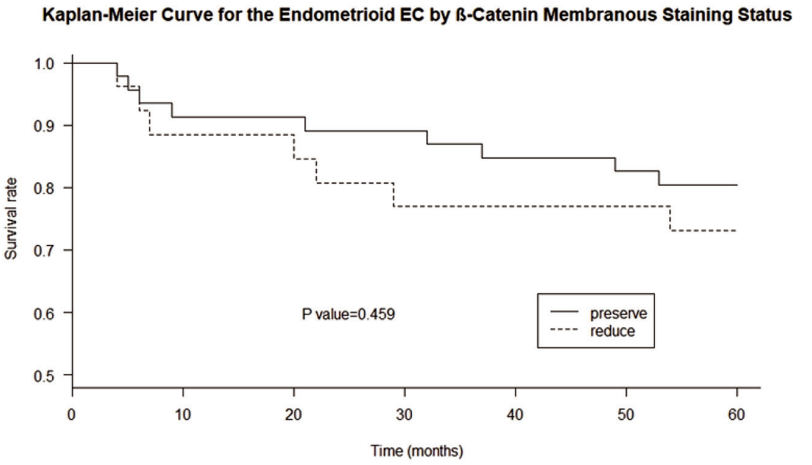

Figure 2. Overall survival of patient with β-catenin membranous expression in 72 type I endometrial cancer.

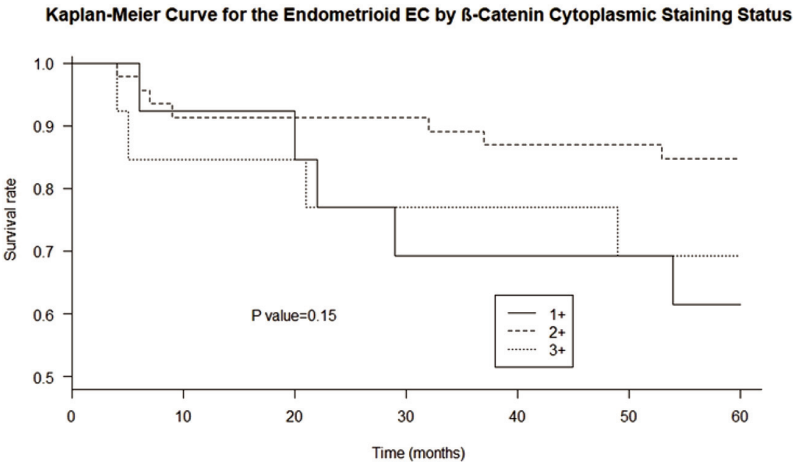

Figure 3. Overall survival of patient with β-catenin cytoplasmic expression in 72 type I endometrial cancer.

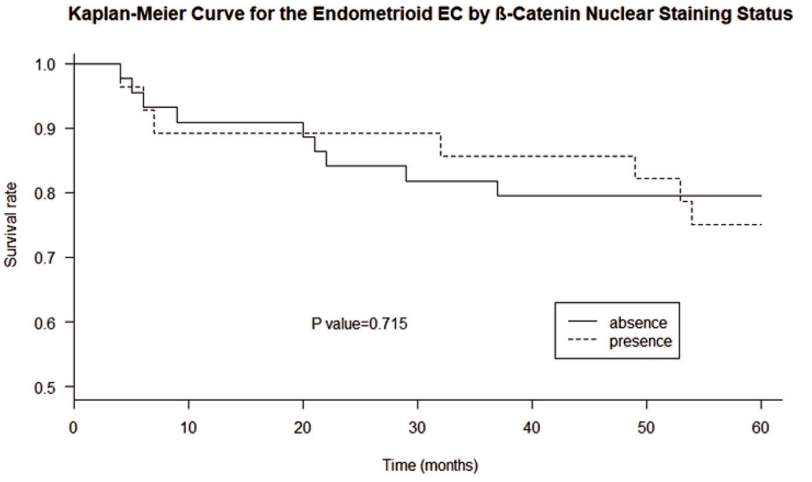

Figure 4. Overall survival of patient with β-catenin nuclear expression in 72 type I endometrial cancer.

Table 2. The association between β-catenin immunohistochemical staining and clinical data

Parameters	β-catenin membranous staining; n (%)			β-catenin cytoplasmic staining; n (%)				β-catenin nuclear staining; n (%)		
	Preserved	Reduced	p-value	1+	2+	3+	p-value	Absence	Presence	p-value
Age (year)			0.337[1]				0.157[1]			0.317[1]
<57	18 (39.1)	14 (53.8)		5 (38.5)	24 (52.2)	3 (23.1)		17 (38.6)	15 (53.6)	
≥57	28 (60.9)	12 (46.2)		8 (61.5)	22 (47.8)	10 (76.9)		27 (61.4)	13 (46.4)	
Stage			0.375[2]				0.629[2]			0.341[2]
1	27 (58.7)	16 (61.5)		7 (53.8)	30 (65.2)	6 (46.2)		25 (56.8)	18 (64.3)	
2	7 (15.2)	1 (3.8)		1 (7.7)	4 (8.7)	3 (23.1)		7 (15.9)	1 (3.6)	
3	11 (23.9)	9 (34.6)		5 (38.5)	11 (23.9)	4 (30.8)		11 (25.0)	9 (32.1)	
4	1 (2.2)	0 (0.0)		0 (0.0)	4 (8.7)	0 (0.0)		1 (2.3)	0 (0.0)	
Grade			0.198[1]				0.037[2]			0.031[1]
1	26 (56.5)	11 (42.3)		3 (23.1)	26 (56.5)	8 (61.5)		18 (40.9)	19 (67.9)	
2	15 (32.6)	8 (30.8)		5 (38.5)	16 (34.8)	2 (15.4)		19 (43.2)	4 (14.3)	
3	5 (10.9)	7 (26.9)		5 (38.5)	4 (8.7)	3 (23.1)		7 (15.9)	5 (17.9)	
Nodal metastasis			1[1]				0.923[2]			0.780[1]
Positive	12 (26.1)	6 (23.1)		4 (30.8)	11 (23.9)	3 (23.1)		12 (27.3)	6 (21.4)	
Negative	34 (73.9)	20 (76.9)		9 (69.2)	35 (76.1)	10 (76.9)		32 (72.7)	22 (78.6)	
Recurrent and/or metastasis			0.309[1]				0.020[2]			1[1]
Yes	8 (17.4)	8 (30.8)		7 (53.8)	7 (15.2)	2 (15.4)		10 (22.7)	6 (21.4)	
No	38 (82.6)	18 (69.2)		6 (46.2)	39 (84.8)	11 (84.6)		34 (77.3)	22 (78.6)	

[1] Chi-squared test, [2] Fisher's exact test

Table 3. The univariate and multivariate cox-regression analysis between β-catenin staining and clinicopathologic factors in type I endometrial cancer

Parameters	Variable	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Age (years)	<57 vs. ≥57	2.72 (0.87 to 8.46)	0.082	7.24 (1.98 to 26.43)	0.002
Stage	1 vs. 2	3.04 (0.55 to 16.62)	0.199	0.95(0.1 to 8.69)	0.967
	1 vs. 3	6.25 (1.92 to 20.35)	0.002	3.67(0.34 to 39.64)	0.285
	1 vs. 4	50.85 (4.76 to 542.0)	0.001	106.43(1.98 to 5710.94)	0.022
Grade	1 vs. 2	4.16 (1.07 to 16.11)	0.038	2.66 (0.67 to 10.47)	0.159
	1 vs. 3	7.85 (1.96 to 31.47)	0.003	12.70 (2.87 to 56.07)	<0.001
Nodal metastasis	Negative vs. positive	6.80 (2.46 to 18.8)	<0.001	13.87 (4.23 to 15.51)	<0.001
Recurrent and/or metastasis	Yes vs. no	6.3 (2.33 to 17.05)	<0.001	6.1 (1.31 to 28.37)	0.021
β-catenin membranous staining	Preserve vs. reduce	1.55 (0.58 to 4.17)	0.382	2.57(0.33 to 19.86)	0.364
β-catenin cytoplasmic staining	1+ vs. 2+	0.39 (0.13 to 1.22)	0.106	6.15(0.47 to 80.71)	0.167
	1+ vs. 3+	1.06 (0.3 to 3.77)	0.925	5.53 (0.44 to 70.20)	0.187
β-catenin nuclear staining	Absence vs. presence	1.20 (0.44 to 3.23)	0.714	1.87 (0.42 to 8.32)	0.410

CI=confidence interval

Discussion

EC is an important problem for women and causes high mortality in its advanced stage. Whilst, the pathogenesis of this tumor is still unclear, WNT or β-catenin abnormality is one of the possible carcinogenesis pathways. Many previous studies have shown discordant results of β-catenin expression in EC and have also shown controversy to the prognostic significance of β-catenin expression[16-19].

In the present study, β-catenin expressions were positive in all cases of endometrioid carcinoma, but in different locations. Moderate to strong β-catenin

cytoplasmic expressions (intensity 2+ and 3+) showed a significant association with low grade EC. In addition, β-catenin nuclear expression is associated with low grade EC. These findings may indicate that the over expression of β-catenin may be a driver of EC. When endometrial cells have mutation of the β-catenin gene, it will increase the concentration of β-catenin proteins in the cytoplasm, and an uptake in the nuclear to increase cellular proliferation. This usually progresses to endometrial hyperplasia and low-grade EC. However, the pathogenesis of high-grade EC and type II EC might have different pathways, such as the p53 mutation pathway, which would explain the loss of β-catenin nuclear expression in high grade EC[33]. The β-catenin immunohistochemical staining might be a useful marker for distinguish between low- and high-grade EC.

FAT1 may be associated with carcinogenesis of type I EC. The function of FAT1 is to inhibit the β-catenin expression, which reduces cell proliferation[20-28]. In the present study, FAT1 immunostaining was negative in all cases. The loss of expression of FAT1 in the present study might be from genetic alteration of the FAT1 gene. The reduction of FAT1 proteins might be the cause of over expression of β-catenin in the tumor cells, found in the present study. However, further molecular study of FAT1 gene expression is needed to confirm this hypothesis.

Survival analysis revealed that FAT1 and β-catenin expressions did not have a significant effect on 5-year OS. Age, stage, grade, nodal status, and recurrent or metastasis status have a significant effect on 5-year OS and might be the independent prognostic factors.

In summary, β-catenin expressions were positive in all cases of type I EC, but different in location. β-catenin cytoplasmic expressions showed a significant association with low grade EC and helpful to predict low recurrent risk. β-catenin had no significant effect on 5-year OS.

Conclusion

β-catenin cytoplasmic expression is associated with grading of EC and may be helpful to predict recurrent disease or metastasis. FAT1 expression is not associated with any clinical factors. β-catenin and FAT1 markers cannot be used to determine the prognosis in EC.

What is already known on this topic?

The previous studies indicated that the expression of β-catenin might be associated with prognosis of EC but this is still a controversy.

What this study adds?

This study showed association between β-catenin cytoplasmic protein expression and grading of type I EC and showed correlation with recurrent disease or metastasis.

Funding disclosure

The funding of this research was supported by the Faculty of Medicine Prince of Songkla University.

Conflict of interest

The authors declare no conflict of interest.

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