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RESEARCH ARTICLE

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Prognostic Value of β -Catenin and LICAM Expressions in Type I Endometrial Carcinoma

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Abstract

Objective: The aim of this study is to evaluate the expression of β -catenin and LICAM in the type I of Endometrial Carcinoma. **Material and Methods:** This study was an analytical study with a cross-sectional design using 49 samples of type I Endometrial Carcinoma. Immunohistochemical method was used to evaluate the expression of β -catenin and LICAM related to two significant prognostic parameters i.e., lymphovascular space invasion (LVSI) and metastases event of type I Endometrial Carcinoma samples. **Results:** From all samples collected, based on the presence of LVSI, there were 17 cases (34.7%) with LVSI and 32 (65.3%) no LVSI. Among them, there were 13 cases that included lymph node or omental samples in type I Endometrial Carcinoma, 5 (38.5%) cases of metastasis, and 8 (61.5%) cases that did not metastasize. The statistical results showed that there was a significant correlation between β -catenin and LICAM expressions examined from tumor cells with lymphovascular space invasion and the presence of metastases in the type I Endometrial Carcinoma ($p < 0.05$). **Conclusion:** This study suggest that the positive expression of β -catenin together with LICAM can participate in the development of tumor cells in type I Endometrial Carcinoma, in its ability to involve lymphovascular space invasion, and metastasize to other sites. Our results indicate that both of β -catenin and LICAM are prominent biomarkers for the prognosis of type I Endometrial Carcinoma.

Keywords: type I Endometrial Carcinoma- β -catenin- LICAM- immunohistochemistry

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Introduction

Endometrial carcinoma is a malignancy of the female genital tract, which is the endometrial lining, with a high attack rate. It is the sixth most commonly occurring cancer in women worldwide and the fifteenth most commonly occurring cancer overall, of which there were over 380,000 new cases in 2018 (Bray et al., 2018). The American Cancer Society estimated that there were 63,230 new cases and 11,350 deaths in 2018 (Siegel et al., 2018). In Western countries, it is also a common malignancy and had the highest rate of endometrial cancer in 2018. Even in developing countries, the incidence of Endometrial Carcinoma is increasing (Bray et al., 2018).

There are two main types of clinicopathology of Endometrial Carcinoma, namely: type I is low grade and estrogen-related, known as Endometrioid Endometrial Carcinoma (EEC) and type II is Nonendometrioid Endometrial Carcinoma (NEEC) which commonly occurs in aged women (Morice et al., 2016). Type I Endometrial Carcinoma has an 80% incidence of endometrial cancer. This malignancy is an endometrial gland neoplasm that gives an image of acinar, papillary, or partially solid formation. It is the same as precursor lesions, that type

I Endometrial Carcinoma can develop from atypical hyperplasia / endometrioid intraepithelial neoplasia, due to excessive "unopposed estrogen" stimulation (Morice et al., 2016; Sanderson et al., 2017).

The role of using biological markers to identify tumor progression to an advanced stage is needed in the initial assessment of the patient to monitor the possibility of tumor aggressiveness and determine the patient's prognosis, which in the end can identify the appropriate and effective management strategies for sufferers.

Besides its known role in several malignancies, β -catenin through Canonical Wnt / β -catenin pathway also plays an important role in Endometrial Carcinoma tumorigenesis, by activating the target gene through stabilization of β -catenin in the nucleus (Coopes et al., 2018; Sanderson et al., 2017).

Nowadays, it has been mentioned by several studies on the existence of L1 cell adhesion molecule (LICAM) as a biomarker against predictive potential and help identify Endometrial Carcinoma with poor outcomes (Bosse et al., 2014). Where LICAM expression shown in Endometrial Carcinoma, is associated with the aggressiveness of histology subtypes, advanced stages, the occurrence of distant metastases, and poor prognosis (Bosse et al., 2014;

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Geels et al., 2016).

There are several prognostic parameters in endometrial cancer, including: age, parity, histological type, histological grade, myometrial invasion, lymphovascular invasion and lymph node metastasis (Sorbe, 2012; la Rubia et al., 2020). In this study, the correlation of β -catenin and L1CAM expressions was evaluated with two prognostic parameters of Endometrial Carcinoma included in histopathology reports that are lymphovascular space invasion (LVSI) and the presence of metastases. LVSI is an important prognostic factor and it is independent of histological grade or depth of myometrial invasion, whereas lymph node metastases are also significant independent prognostic factors for poor survival (Sorbe, 2012).

Materials and Methods

This study was conducted at the Anatomical Pathology Laboratory, Hasanuddin University Hospital Makassar. The population of this study as the inclusion criteria was resection tissue from endometrium and diagnosed as type I Endometrial Carcinoma grade 1, grade 2, and grade 3, from hematoxylin and eosin staining slides, including LVSI and metastases status contained in histopathological reports in the hospital.

There were 49 samples that met the inclusion criteria consisting of 17 cases of type I Endometrial Carcinoma with LVSI, 32 cases with no LVSI and of these there were 13 samples that included either lymph node or omental tissue.

Immunohistochemical procedures were performed using β -catenin monoclonal antibodies (catalog No. GTX34442, dilution 1:50; Gene Tex Laboratory) and L1CAM polyclonal antibody (catalog No. A00729-1, dilution 1:50; Boster Biological Technology). Immunohistochemical staining results were evaluated using a light microscope by two pathologists and researchers. L1CAM and β -catenin immunoreexpression are expressed in semi-quantitative estimates with a scoring system, namely:

Evaluation of L1CAM expression, was evaluated by presentation of stained areas on tumor cell membranes: There was no colored area (score 0); Colored area <10% (score 1); Colored area 11-50% (score 2); Colored area >50% (score 3) and score interpretations are divided into two categories, namely: 0-1 score = negative expression;

and 2-3 scores = positive expression (Zeimet et al., 2013).

Scoring of β -catenin expression in the cytoplasm and cell nucleus, by evaluating the intensity of β -catenin color: There are no normal/stained epithelial cells (score 0); Weak (score 1); Moderate (score 2); Strong (score 3); and evaluation the percentage of the colored area: There is no colored area (score 0); Colored area <10% (score 1); Colored areas are 10-25% (score 2); Colored areas 25-50% (score 3); and Colored area > 50% (score 4); then summed up from the intensity scores and colored area presentations, and will get a total score range of 0-7, which in turn the interpretations are divided into two categories as follows: 0-4 = negative expression; and 5-7 = positive expression (Florescu et al., 2016).

This study was an analytical study with cross-sectional design. Bivariate analysis in the form of Chi-square test was used.

Results

Table 1 shows the characteristics of the samples in the study. The average age of type I Endometrial Carcinoma patients is 51 years old. The age of patients younger than 50 years old were 21 (42.9%), and older than 50 years old were 28 (57.1%). The sample distribution based on the diagnosis of histopathological grading showed that there were 15 cases of type I Endometrial Carcinoma grade 1 (30.6%), 20 cases grade 2 (40.8%), and 14 cases grade 3 (28.6%). Meanwhile, of all the samples collected, there were only 13 cases that sent samples in the form of lymph node and omentum for evaluation of metastases presence in type I Endometrial Carcinoma : 5 metastatic cases, and 8 other cases without metastases.

Microscopic evaluation of β -catenin and L1CAM expressions with immunohistochemical staining showed positive expression of β -catenin in the nucleus and cytoplasm, whilst L1CAM positive expression appears on the cell membrane (Figure 1-A,1-B).

Microscopic evaluation of the expression of β -catenin and L1CAM on tumor cells in the lymphovascular space invasion area, both showed positive expression in almost all type I Endometrial Carcinoma samples with lymphovascular space invasion (Figure 2-A,2-B).

In this study, a microscopic evaluation of β -catenin and L1CAM expressions by immunohistochemical staining of type I Endometrial Carcinoma that metastases to lymph

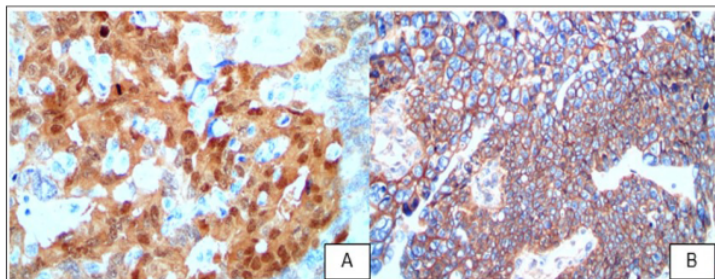


Figure 1. A). Positive expression of β -catenin on the nucleus and cell cytoplasm of type I endometrial carcinoma Grade 2 (200x). B). Positive expression of L1CAM on cell membranes of type I endometrial carcinoma Grade 2 (200x).

Table 1. Characteristics of the Sample

Characteristics	Number	%
Age	(n = 49)	
< 50	21	42.9
≥ 50	28	57.1
Mean	51.1	51.1
Histopathological Grade (FIGO)		
Grade 1	15	30.6
Grade 2	20	40.8
Grade 3	14	28.6
LVSI		
Yes	17	34.7
No	32	65.3
	(n = 13)	
Lymph node or omental metastases		
Yes	5	38.5
No	8	61.5

node or omentum was also carried out. Microscopic examination of all samples revealed positive expression of β-catenin and L1CAM (Figure 2-C,2-D).

Table 2 shows the results of statistical tests of β-catenin and L1CAM expression scores with lymphovascular space invasion correlation. It results in $p = 0.0001$ ($p < 0.05$), which means there are significant differences between β-catenin and L1CAM expression scores with the presence of lymphovascular space invasion of type I Endometrial Carcinoma. The table indicates that positive expression of both β-catenin and L1CAM influences the level of lymphovascular invasion. Compared to one or two proteins with a negative expression, which show less influence on level of lymphovascular space invasion.

Table 2. β-catenin and L1CAM Expressions in Type I Endometrial Carcinoma based on the Presence of Lymphovascular Space Invasion (LVSI)

β-catenin and L1CAM expression interpretations	LVSI		p-value
	Yes	No	
β-catenin positif and L1CAM positive	14 (82.4%)	0 (0%)	<0.05*
β-catenin positif and L1CAM negative	2 (11.8%)	0 (0%)	
β-catenin negative and L1CAM positive	0 (0%)	0 (0%)	
β-catenin negative and L1CAM negative	1 (5.9%)	32 (100%)	
Total no. (%)	17 (100%)	32 (100)	

LVSI, Lymphovascular Space Invasion; * Chi-Square Test

The results of the statistical tests shown in table 3 is the correlation between the β-catenin and L1CAM expression scores with the occurrence of metastasis, which shows the

Table 3. β-catenin and L1CAM Expressions in Type I Endometrial Carcinoma based on the Presence of Lymph Node or Omental Metastases

β-catenin and L1CAM expression interpretations	Metastases		p-value
	Metastases	No metastases	
β-catenin positif and L1CAM positive	4 (80.0%)	0 (0%)	<0.05*
β-catenin positif and L1CAM negative	0 (0%)	0 (0%)	
β-catenin negative and L1CAM positive	1 (20.0%)	0 (0%)	
β-catenin negative and L1CAM negative	0 (0%)	8 (100%)	
Total no. (%)	5 (100)	8 (100)	

*, Chi-Square Test

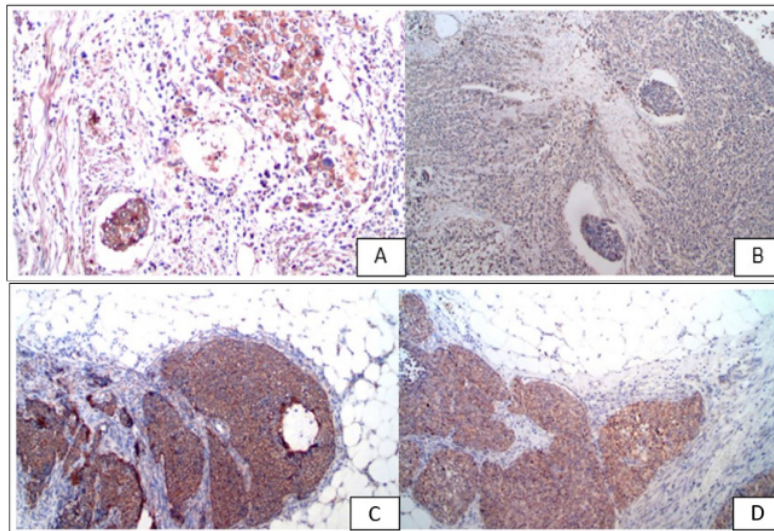


Figure 2. A). β-catenin positive expression of tumor cells in the area of Lymphovascular invasion (200x). B). L1CAM positive expression of tumor cells in the area of lymphovascular invasion (100x). C). Positive expression of β-catenin in type I endometrial carcinoma that metastasizes to the omentum (100x). D). L1CAM positive expression in type I endometrial carcinoma metastasizes to the omentum (100x).

16 results of $p = 0,0001$ ($p < 0.05$). This indicates that there are significant differences between β -catenin and LICAM expression scores with the presence of metastatic events in type I Endometrial Carcinoma. The table also shows that positive expression of both β -catenin and LICAM strongly influence the metastatic events. Compared to one protein with a negative expression, that more less influences the presence of metastases.

Discussion

28 Endometrial Carcinoma patients if detected at an early stage will have a better prognosis with a life expectancy of around 85% in 5 years (Notaro et al., 2016; Voller Putten et al., 2017). Therefore, the ability to detect patients with a high risk of Endometrial Carcinoma can have a major impact on sufferers. Recent studies revealed currently promising prognostic immunohistochemistry markers whether these markers can help to assist the gynecological oncology surgeon selecting the adequate surgical extent, there has been evaluated the correlation with LVSI as the important markers for adjuvant treatment strategy decision (Veinberger et al., 2019).

The finding in this study demonstrate there was a correlation between positive expressions of β -catenin and LICAM with LVSI in type I Endometrial Carcinoma. Further in this study, an association between the positive expression of β -catenin and LICAM with the presence of metastases in the lymph nodes and omentum was found.

Previous studies have reported that positive expression of LICAM immunohistochemical examination results has a strong relationship with the depth of myometrial invasion and the presence of lymphovascular invasion in Endometrial Carcinoma (Dellinger et al., 2016; Van Gool et al., 2016). Another study also stated that there was a relationship between LICAM expression and the presence of lymphovascular invasion in Endometrial Carcinoma. In addition, there is a close relationship between LICAM expression with the involvement or incidence of lymph nodes metastases (de Freitas et al., 2018; Geels et al., 2016). Whereas a previous research study conducted by Flores et al., (2016), suggested that there was a relationship between β -catenin expression and tumor staging, degree of differentiation, and myometrial invasion. Instead, it was found that there were no association between β -catenin expression and metastatic involvement to lymph nodes. On the other hand, there was another study that had suggested that Endometrial Carcinoma patients with β -catenin mutations were significantly more likely to have tumors with pathological characteristics that generally had a lower clinical risk of recurrence (lower histologic grade, less incidence of deep myometrial invasion, and less incidence of lymphovascular space invasion (Kurnit et al., 2017)).

LICAM induces EMT in several cancers, including Endometrial Carcinoma. Previous studies have reported that LICAM and β -catenin, both have prognostic values and can be used as prognostic markers in patients with Endometrial Carcinoma, mentioned that LICAM upregulation events in Endometrial Carcinoma were

induced by β -catenin and TGF β / SLUG (Pfeifer et al., 2010; Giordano and Cavallaro, 2020). Further explained, LICAM is the target gene of the Wnt / β -catenin signaling pathway, where β -catenin accumulation in the nucleus shows the same location as LICAM. Therefore, it explains that the transcriptional β -catenin / TCF-LEF complex is assumed to be a direct regulator of LICAM expression (Pfeifer et al., 2010; Giordano and Cavallaro, 2020). It was also stated that tumor cells from Endometrial Carcinoma present the process of Epithelial-mesenchymal transition (EMT) induced by TGF- β , which then up-regulates LICAM with vimentin, both of which down-regulation of E-Cadherin, which this mechanism depends on the transcription factor Slug (Zeimet et al., 2013). Pfeifer et al., (2010) conducted a research study in which the LICAM gene had 2 functionally active promoters namely Slug and β -catenin which are involved in the transcriptional regulation of LICAM. Pfeifer carried out the LICAM gene analysis in detail with the PCR technique, and concluded that the regulation of LICAM expression in tumor cells in Endometrial Carcinoma was carried out by two different promoter regions, which Slug transcription factors were the relevant transcription factors in this LICAM regulation.

Several previous studies mentioned above support the findings that are found in this study, where there is a significant correlation between β -catenin and LICAM expression in type I Endometrial Carcinoma that associates with lymphovascular space invasion and metastasis events.

In conclusion, the results of this study suggest that there is a tendency for overexpression of β -catenin along with LICAM which can increase the progressivity of tumor cells in type I Endometrial Carcinoma in its ability to involve lymphovascular space invasion and metastases to other tissues. Thus, both β -catenin and LICAM have prognostic values and can be used as prognostic markers in patients with Endometrial Carcinoma.

Author Contribution Statement

The methodology was planned and designed by YM, RM and UM; YM, BN, MHC and M were involved in data gathering, processing, and reporting. UM and YM, conducted a comprehensive conceptual and editorial evaluation; the finalization of the article was amended and approved by all of the contributors.

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Study Approval

39 The research committee of the Faculty of Medicine at Hasanuddin University approved this project.

Ethical approval

The Ethics Committee of the Faculty of Medicine

granted informed consent for this study (Protocol #UH18060363, Archive No. 442/H4.8.4.5.31/PP36-KOMETIK).

Availability of Data

On reasonable request, the associated author will release the datasets used in this work.

Conflict of Interest

All contributors report having no competing interests.

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