Oncology
and Radiotherapy ©
Vol.14 Iss.5:001-006 • RESEARCH ARTICLE

Expression of L1CAM and Ki-67 in endometrial cancer of Egyptian females: clinical impact and survival

Fatma Gharib[1], Dareen Abd Elaziz Mohamed[2], Walid Ahmed Almorsy[1]

[1] Department of Clinical Oncology, Tanta University, Egypt
[2] Department of Pathology, Tanta University, Egypt

SUMMARY

Introduction: Endometrial adenocarcinoma is characterized by a good prognosis. However, the disease response shows a significant heterogeneity. Treatment of Endometrial Cancer (EC) is still based on clinico-pathological parameters, which have limited role in risk stratification. There is a need for more determinant markers, such as L1 Cell Adhesion Molecule (L1CAM), to identify patients at higher risk of relapse and tailor a more convenient treatment. L1CAM has a capacity to enhance cell motility and promote tumour invasion in different malignancies. In Egypt, the incidence rate of EC is growing over time. Especially in Elgharbiah governorate (home of this study). L1CAM expression and Ki-67 was reported and compared with other clinico-pathological criteria. Method: Seventy-six female patients of endometrial carcinomas were involved in this prospective study. The patients were treated and followed up at Tanta University Hospitals in the period between January 2015 to April 2019. L1CAM expression and Ki-67 was detected by immuno-histochemical exam and compared with other clinico-pathological criteria. Survival was assessed and compared by Kaplan-Meier curves and log-rank test.

Results: Positive L1CAM expression was detected in 17 patients (22.4%) and was significantly correlated with unfavourable prognostic factors such as higher stage and grade, lympo-vascular invasion, non-endometroid type and ki-67 Univariate analysis revealed that: positive L1CAM; higher tumour grade; high stage; and non-endometrioid type were significantly associated with shorter Disease-Free Survival (DFS) but no significant correlation was detected between ki-67 and DFS. Positive L1CAM remained statistically significant with DFS in multivariate analysis (p=0.045; 95%CI (1.028:11.17); HR=3.38).

Conclusion: Our study indicates that L1CAM expression and Ki-67 are significantly associated with poor tumour characteristics. L1CAM is significantly associated with shorter disease-free survival and may be a helpful tool as a part of a simple clinical molecular classification for EC

Key words: L1CAM, endometrial cancer, Ki67

Address for correspondence:

Fatma Gharib, Department of Clinical Oncology, Tanta University, Egypt, email: dfatma1980@yahoo.com

Word count: 2958 **Tables:** 04 **Figures:** 03 **References:** 29

Received: - 24 August, 2020

Accepted: - 15 September, 2020

Published: - 25 September, 2020

INTRODUCTION

Uterine cancer is the most common gynecological malignancy in the western countries and the second prevalent cancer in developing countries [1]. The incidence rate of uterine cancer is growing over time in Egypt especially in El-Gharbiah province of Egypt [2, 3].

Treatment of Endometrial Cancer (EC) is largely based on clinicopathological parameters, such as histological type, tumour stage and grade [4-6]. Several studies clarified that Clinicopathological features had limited role in risk stratification particularly for high-grade tumours [7-9].

The Proactive Molecular Risk Classifier (ProMisE) is an important classification for endometrial cancer. ProMisE is focusing on molecular parameters which is prognostically significant in endometrial carcinomas. The current WHO system is principally based on histomorphological parameters [10-12]. The subtypes of promise are DNA POLE exonuclease domain mutant, mismatch repair deficiency, *p53* wild type and abnormal type [12].

Despite these advancement in EC classification, new markers are still required to recognize a subgroup at risk of relapse and tailor treatment (adjuvant radiotherapy, chemotherapy and/or need for advanced surgery). The L1-Cell Adhesion Molecule (L1CAM) is a transmembrane protein of the immunoglobulin family. Several studies have evaluated the prognostic significance of L1CAM in EC and other tumours [13, 14]. L1CAM seems to be a predictive factor of lymph node involvement, associated with unfavourable prognostic factors and promotes tumour cell proliferation, invasion and metastasis [15-18].

In endometrial tumour, Detection of L1CAM by immunohistochemical exam could be able to identify subgroups of more aggressive tumours with high risk of distant metastases and pelvic lymph-node involvement [19-22].

AIM

The objective of this study was to determine the clinical impact of L1CAM and Ki-67 and their association with other risk factors and survival in endometrial Adenocarcinoma.

PATIENTS AND METHODS

Seventy-six cases of endometrial carcinomas were involved in this prospective study. Treatment and follow-up of patients

were done at Clinical Oncology Department through the period from January 2016 to April 2020.

The entire patients underwent total hysterectomy and bilateral salpingo-oophorectomy. The histological diagnosis, tumour stage, and grading were classified by International Federation of Gynecology and Obstetrics (FIGO) 2009 criteria. The local ethical committee approved this study and all patients gave an informed consent.

The following histological criteria were studied: age, stage, grade, status of Lymphovascular Invasion (LVSI), histological subtypes and Ki-67. Exclusion criteria included Stage IV EC, Presence of synchronous or metachronous second malignancies and incomplete surgical staging.

Therapeutic approach and follow-up

Adjuvant radiotherapy: Patients with stage II and stage I with high risk features LVSI, older age, outer half myometrial invasion, grade 2 or 3 differentiation) received adjuvant radiotherapy (50 Gy given in 2.0 Gy fractions over 5-6w). We use four-field beam arrangement for pelvic irradiation, to confirm the entire draining lymph nodes and the vaginal cuff are involved in the treatment planning. Three D Conformal Radiotherapy (3DCRT) was given 6 weeks after surgery using Eclipse version 7.0 (Varian Medical Systems).

Adjuvant chemotherapy: Patients with high-risk endometrial cancer (stage III, grade 3, non-endometroid carcinoma) received carboplatin AUC=6/3 weeks and paclitaxel 80 mg/m^2 IV weekly for six cycles.

Follow up of the patients was performed every 3 months in the first 3 years then every 6-months.

Immunohistochemistry and staining

Immunohistochemical staining on 4-µm sections of formalin-fixed paraffin embedded tissue after deparaffinization, ethanol was used for rehydration and tris-buffered saline, tissues were incubated in retrieval solution pH 9.0 in a steamer. L1CAM was detected using a primary mouse monoclonal antibody (Thermo Fisher Scientific clone UJ127dilution 1:50, Waltham, MA). Ki-67 monoclonal mouse, DAKO, Carpinteria, CA), at a dilution of 1:100. Sections were counterstained with Mayer haemalaun solution finally, slides were covered (Figures 1 and 2).

According to percent of positive tumour cells, L1CAM expression was scored (score 0=0%, score 1=1-10%, score 2=> 10-50% and score 3=>50%). If ≥ 10% (scores 2 and 3) of epithelial tumour cells showed membranous L1CAM staining, tumours were determined L1CAM positive [23].

For manual scoring of ki-67 percentage, the percent of positive stained nuclei within 3 high-powered fields (x40 magnification) selected across the tumour randomly was calculated and at least

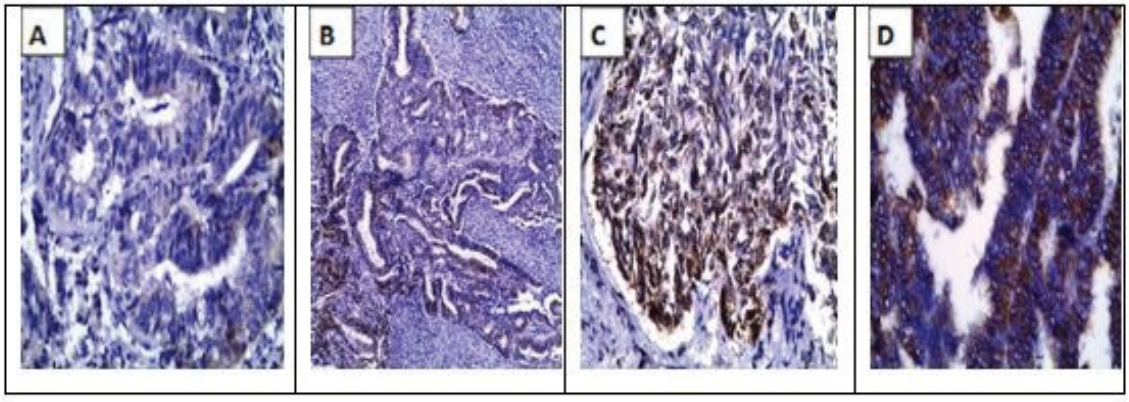

Fig. 1. (A) well differentiated endometrioid carcinoma with weak positive expression of L1CAM immunostaining (X400); (B) Moderately differentiated endometrioid carcinoma with moderate positive expression of L1CAM X200; (C) Poorly differentiated endometrioid carcinoma showing strong positive expression of L1CAM (X400); (D) papillary serous adenocarcinoma showing strong positive expression of L1CAM (X400)

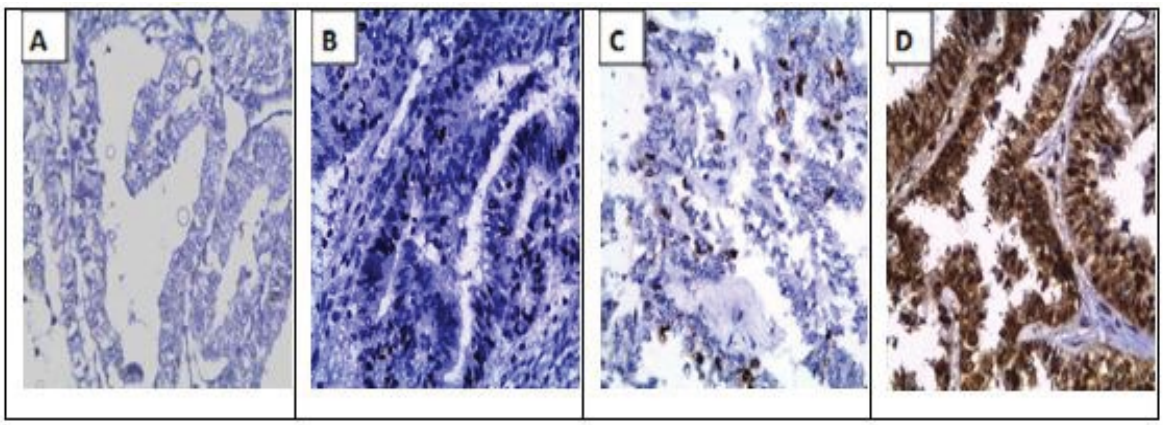

Fig. 2. (A) well differentiated endometrioid carcinoma showing low ki67 expression (X400); (B) moderately differentiated endometrioid carcinoma showing intermediate KI67 (X400); (C) poorly differentiated endometrioid carcinoma showing high KI67 (X400); (D) papillary serous adenocarcinoma showing high KI67 (X400)

1000 nuclei were counted. According to the ki-67 labelling index of ≤ 15%, 16%-30%, and>30%, respectively, tumours were classified as low, intermediate, and high proliferating.

For statistical analysis, we grouped the patients according to the Ki-67 labelling index to Ki-67 ≤ 15% (low score) and Ki-67 >15% (high score)

Statistical analysis

Associations between L1CAM, Ki-67 and other clinicopathological criteria were analysed. Disease free survival is defined as time interval after primary treatment to recurrence or death. Kaplan-Meier with log rank test was used for estimation of survival rate by SPSS software, (version 21.0). P-value<0.05 considered statistically significant.

RESULTS

This prospective study included 76 patients with endometrial adenocarcinoma. The median age was 58 years (range 31-72). Depth of myometrial infiltration>1/2 was reported in 43 patients (56.6%). Thirty-one patients (40.8%) had Stage II and forty-three patients (56.6%) had grade 2 tumour. About 54% of patients were obese (BMI ≥ 30 kg/m²). Clinicopathological features were gathered in (Table 1).

Seventeen patients (22.4%) had positive L1CAM expression. There was a significant association between positive L1CAM expression and cervical involvement (p=0.013), high grade and stage (p=0.001 and p=0.021 respectively), LVSI (p<0.001), non-endometroid type (p<0.027) and ki-67 (p= 0.003) (Table 2).

High ki-67 was reported in 25% of cases and significantly correlated with myometrial invasion>1/2 (p=0.011), high tumour grade (p=0.027), LVSI (p=0.009) and BMI>30 kg/m² (p=0.024) and non-endometroid type (p<0.001) (Table 3).

Disease free survival

The estimated mean Disease-Free Survival (DFS) was 41 months. The 3-year DFS rate

was 82.6%. Correlation of DFS to the other prognostic features was clarified (Figure 3).

Univariate analysis revealed significant association between positive L1CAM, high tumour grade, high stage, non-endometrioid type and short disease-free survival. The association between high ki-67 and DFS was statistically insignificant.

Tumour stage and positive L1CAM remained significantly associated with DFS in multivariate analysis p=0.045; 95% CI (1.028:11.17); HR=3.38) and p=0.048; 95% CI (1.009:7.101); HR=2.676) respectively (Table 4).

Tab. 1. Clinico-pathologic characteristics

Clinico-pathologic characteristics	(n=76)	%
Age group		
≤ 58	30	39.5
>58	46	60.5
Myometrial invasion		
≤ 1/2	33	43.4
>1/2	43	56.6
Cervical involvement		
No	54	71.1
Yes	22	28.9
Stage		
I	22	28.9
II	31	40.8
III	23	30.3
Tumour grade		
G1	8	10.5
G2	43	56.6
G3	25	32,9
Lymphovascular invasion		
Absent	60	78.9
Present	16	21.1
Histological type		
Endometrioid	56	73.7
Non endomet rioid	20	26.3
BMI		
≤ 30 kg/m²	35	46.1
>30 kg/m²	41	53.9
Ki 67		
<15%	57	75
≥15%	19	25
L1CAM		
Positive	17	22.4
Negative	59	77.6

Tab. 2. Correlation of Clinico-pathologic features and L1CAM

Patients characteristics (N=53)	L1CAM				p
	Negative (59)		Positive (17)		
	no	%	no	%	
Age group					
≤ 58	25	42.4	5	29.4	0.335
>58	34	57.6	12	70.6	
Myometrial invasion					
<1/2	36	61	7	41.2	0.146
>1/2	23	39	10	58.8	
Cervical involvement					
No	46	78	8	47.1	0.013*
Yes	13	22	9	52.9	
Stage					
I	21	35.6	1	5.9	
II	24	40.7	7	41.2	0.021*
III	14	32.7	9	52.9	
Tumour grade					
G1	8	13.6	0	0	
G2	38	64.4	5	29.4	0.001*
G3	13	22	12	70.6	
Lymphovascular invasion					
Absent	54	91.5	6	35.3	<0.001*
Present	5	8.5	11	64.7	
Histological type					
Endometrioid	47	79.7	9	52.9	0.027*
Non endometrioid	12	20.3	8	47.1	
BMI					
≤ 30 kg/m^2	28	47.5	7	41.2	0.647
>30 kg/m^2	31	52.5	10	58.8	
Ki 67					
<15%	49	83.1	8	47.1	0.003*
≥ 15%	10	16.9	9	52.9	

*p value less than 0.05

Tab. 3. Correlation of Clinico-pathologic characteristics and Ki67

Patients characteristics (N=76)	Ki-67				p
	<15 (%)		≥ 15 (%)		
Age group					
≤ 58	23	40.4.	7	36.8	0.786
>58	34	59.6	12	63.2	
Myometrial invasion					
≤ 1/2	37	64.9	6	31.6	0.011*
>1/2	20	35.1	13	68.4	
Cervical involvement					
No	39	68.4	15	78.9	0.381
Yes	18	31.6	4	21.1	
Stage					
I	18	31.6	4	21.1	0.403
II	24	42.1	7	36.8	
III	15	26.3	8	42.1	
Tumour grade					
G1	7	12.3	1	5.3	0.027*
G2	36	63.2	7	36.8	
G3	14	24.6	11	57.9	
Lymphovascular invasion					
Absent	49	86	11	57.9	0.009*
Present	8	14	8	42.1	
Histological type					
Endometrioid	50	87.7	6	31.6	<0.001*
Non endometrioid	7	12.3	13	68.4	
BMI					
≤ 30 kg/m^2	22	38.6	13	68.4	0.024
>30 kg/m^2	35	61.4	6	31.6	
L1CAM					
Positive					
Negative					

*p value less than 0.05

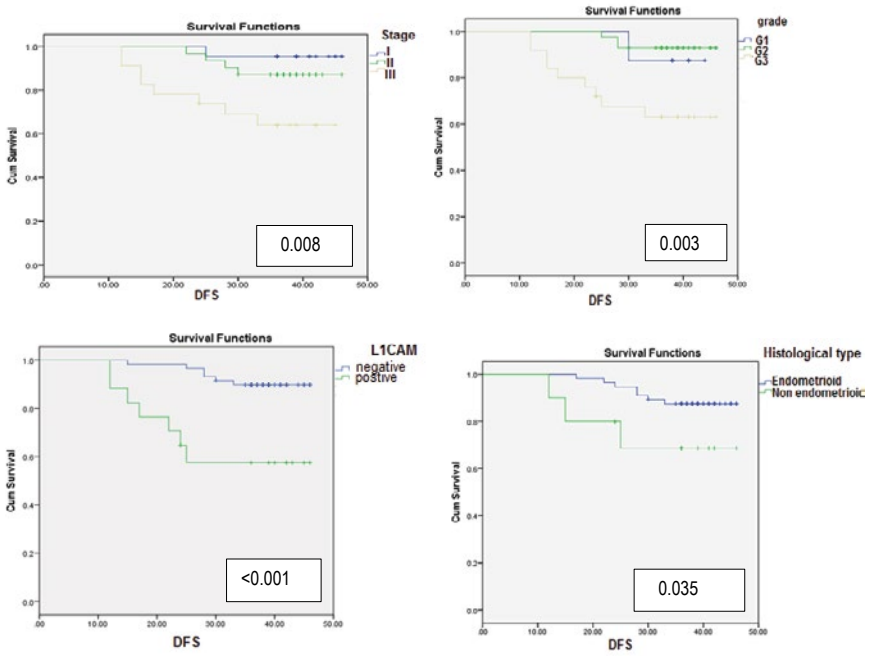

Fig. 3. Disease free survival in relation to prognostic features

Tab. 4. The effects of clinicopathologic features on disease free survival rate by univariate and multivariate analysis

		Univariate		Multivariate		
	3-year DFS (%)	**95% CI**	**p**	**HR**	**95% CI**	**p**
Age group						
≤ 57	86.7	0.502:5.303	0.408			
>57	80.1					
Myometrial invasion						
≤ 1/2	88.6	0.130:1.216	0.092			
>1/2	74					
Cervical involvement						
No	83	0.318:3.351	0.958			
Yes	81.6					
Stage						
I	95.5					
II	87.1	1.366: 7.805	0.008*	2.676	1.009:7.101	0.048*
III	73.9					
Tumour grade						
G1	87.5			1.544	0.454:5.253	0.487
G2	93	1.423: 11.660	0.003*			
G3	63					
Lymphovascular invasion						
Absent	86.3	0.918:8.598	0.058			
Present	68.8					
Histological type						
Endometrioid	87.5	1.022:9.110	0.035*	2.206	0.652:7.464	0.203
Non endometrioid	86.6					
BMI						
≤ 30 kg/m^2	90.1	0.846:8.920	0.078			
>30 kg/m^2	74.2					
Ki 67						
<15%	85.9	0.737:6.920	0.141			
≥ 15%	72.2					
L1CAM						
Positive	57.5	1.987:17.958	<0.001*	3.389	1.028:11.17	0.045*
Negative	89.8					

*p value less than 0.05

DISCUSSION

The ability of L1CAM to enhance cell motility and promote invasiveness has been investigated in different cancers including gastric, endometrial, breast and colorectal carcinoma [24]. Detection of L1CAM in endometrial carcinoma can predict clinical outcome, risk of distant recurrences and risk of pelvic nodal involvement especially in early stages of EC [25].

In multiple previous trials, the correlation between L1CAM expression and other clinicopathological features such as LVSI, deep myometrial invasion, and cervical invasion was conflicting [26, 27].

In our trial, L1CAM expression was significantly correlated with high tumour grade, advanced FIGO stage, LVI and non-endometrioid histology in agreement with Geels et al. [26] and Dellinger et al. [19].

Univariate and multivariate analysis revealed that patients with L1CAM positive tumours were associated with shorter disease survival. Our results are matched with Geels et al. who reported that patients with L1CAM positive tumours had a worse 5-year progression free survival rate compared to the L1CAM-negative group (55.6% and 83.3 % respectively p=0.01) [26].

Ki-67 is a cell proliferation marker, that reflects the degree of proliferation of malignant cells, tumour invasion, metastasis and prognosis of various malignancies. In endometrial carcinoma, there is association between Ki-67 score and other pathological variables such as depth of myometrial invasion, stage, grade and outcome [28, 29].

We found a significant correlation between high Ki-67 and tumour grade, LVI and non-endometroid type nearly like L1CAM expression. Ki-67 is also associated with myometrial invasion and high BMI.

So, a comprehensive combination of both L1CAM and Ki-67 is of great importance for detection of EC prognosis and can identify patients needing aggressive treatment even in early stage endometrioid type.

CONCLUSION

L1CAM is significantly associated with clinicopathological features predicting poor outcome. Moreover, L1CAM can be considered as helpful test in the identification of endometrioid carcinoma with poor prognosis. Future large studies are required to determine the association between L1CAM expression and possibility of tumour invasion and metastasis.

CONFLICT OF INTEREST

The authors declare that they have no interest conflict.

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