Stratification of Endometrial Carcinoma Patients Using Immunohistochemistry for Better Surgical Planning and Prognosis

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Original Article

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ABSTRACT

Background: Endometrial cancer (EC) incidence is rising, necessitating precise risk stratification for optimal therapy. ESMO-ESGO-ESTRO guidelines aid in determining lymph node dissection and adjuvant treatment. New prognostic markers are needed for accurate patient stratification. Our study aims to utilize preoperative IHC analysis of L1CAM, ER, PR, and p53 to enhance surgical planning and outcomes in EC patients.

Patients and Methods: A retrospective-prospective cohort-study was conducted at multiple departments in Zagazig University Hospitals. Sixty patients with confirmed endometrial carcinoma scheduled for surgery between January 2019 and March 2022 were included. IHC staining for ER, PR, L1CAM, and p53 was performed and correlated with ESMO-ESGO-ESTRO guidelines and lymph node status.

Results: Statistically significant correlations were found between IHC markers and histopathological findings, with abnormal-P53 and L1CAM not correlating with histological type and grade. Positive-ER/PR expression had a 3-year OS of 93.5%, while ER-negative patients had 64.3% ($p = 0.002$). Abnormal-P53 was linked to poorer OS (54.5%) compared to normal (93.9%) ($p<0.001$). L1CAM-negative patients had a better OS (94.2%) vs. L1CAM-positive (37.5%) ($p<0.001$). ER/PR-negative, abnormal-P53, and L1CAM-positive patients had poorer PFS (35.7%, 18.2%, 12.5%) ($p<0.001$). Poorer RFS was seen in P53-abnormal, L1CAM-positive, ER/PR-negative, and high-risk ESMO-ESGO-ESTRO subgroups ($p<0.001$). High-risk ESMO-ESGO-ESTRO subgroups were independently associated with reduced PFS, as were L1CAM-positive and P53-abnormal patients ($p=0.002$, $p=0.07$).

Conclusion: We concluded that pre-operative IHC-biomarkers (L1CAM and P53) could be used as refinement for lymph node directed surgery and also adjuvant selective treatment by being integrated in the ESMO-ESGO-ESTRO risk classification.

Key Words: Cancer; endometrial; L1CAM; P53.

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INTRODUCTION

Endometrial carcinoma (EC) is the commonest female cancer in developed countries with a growing incidence due to advanced life expectancy and obesity[1]. Generally, early-diagnosed patient shows a good prognosis. Yet, some cases with even early-stage disease show adverse prognosis[2]. Endometrial carcinoma is classified histologically as endometrioid type that carry a better outcome and non-endometrioid type with unfavorable prognosis[3].

Risk classification systems that are currently used depend on clinic-pathological parameters and this direct the primary and adjuvant therapy. Different systems are commonly used: The European Society for Medical Oncology - European Society of Gynecological Oncology -European Society for Radiotherapy & Oncology (ESMO-ESGO-ESTRO), Gynecologic Oncology Group (GOG) criteria and Post-operative Radiation Therapy for Endometrial Carcinoma (PORTEC)[1,4,5,6]. These systems stratify patients as 'low, low-intermediate, intermediate,

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high-intermediate, high or advanced/metastatic' relying on the histology, grade, stage of tumor and age[4,5,6]. The ESMO- ESGO-ESTRO risk classification could determine lymph node (LN) surgical excision pre-operatively, and also after surgery to determine adjuvant therapy.

Generally, hysterectomy with bilateral salpingo-oophorectomy is done for low-risk group patients, whereas higher-risk group patients would necessitate more aggressive procedures, as para-aortic or pelvic lymphadenectomy with or without adjuvant chemo and radiotherapy. However, these procedures carry significant hazards, with risk of thrombosis, hemorrhage and lymphedema[7]. So, it is important to identify new prognostic markers for better stratification of patients for avoiding under- or over treatment of patients with EC.

The L1 neuronal cell-adhesion molecule (L1CAM) has gained attention recently as an important prognostic and potentially therapeutic target in endometrial carcinoma as well as other tumors[8]. Many studies proved its prognostic utility in EC cohorts[9,10,11]. Its up regulation was proved as a major effector for motility of cancer cells and closely related to epithelial to mesenchymal transition (EMT) process. Cancers showing EMT tend to be presented at an advanced stage with a biologically aggressive behavior[12].

Over-expression of L1CAM, negative expression of estrogen receptors (ER) and/or progesterone receptors (PR) are linked with adverse prognostic outcomes and a higher risk of recurrence and mortality[13]. Also, mutations of p53 are associated with expression of L1CAM, but not universally[14].

In this study, we aim to better surgical planning for improving outcome of endometrial carcinoma patients using preoperative assessment of L1CAM, ER, PR, and p53 in patient stratification and its relation to the post-operative ESMO-ESGO-ESTRO risk groups; also, their correlation with pathological parameters.

PATIENTS AND METHODS

Patients

This is a retrospective-prospective cohort study; carried out in pathology, obstetrics and gynecology, general surgery, clinical oncology and medical oncology departments of faculty of medicine, Zagazig university hospitals. The study included 60 patients who will be operated for histologic confirmed EC, from January 2019 to March 2022. The Zagazig university ethical committee approved the study (ZU-IRB#:10903) clinical trial.gov registration (NCT 06148129) and informed written consent was obtained from included cases.

Preoperative Imaging

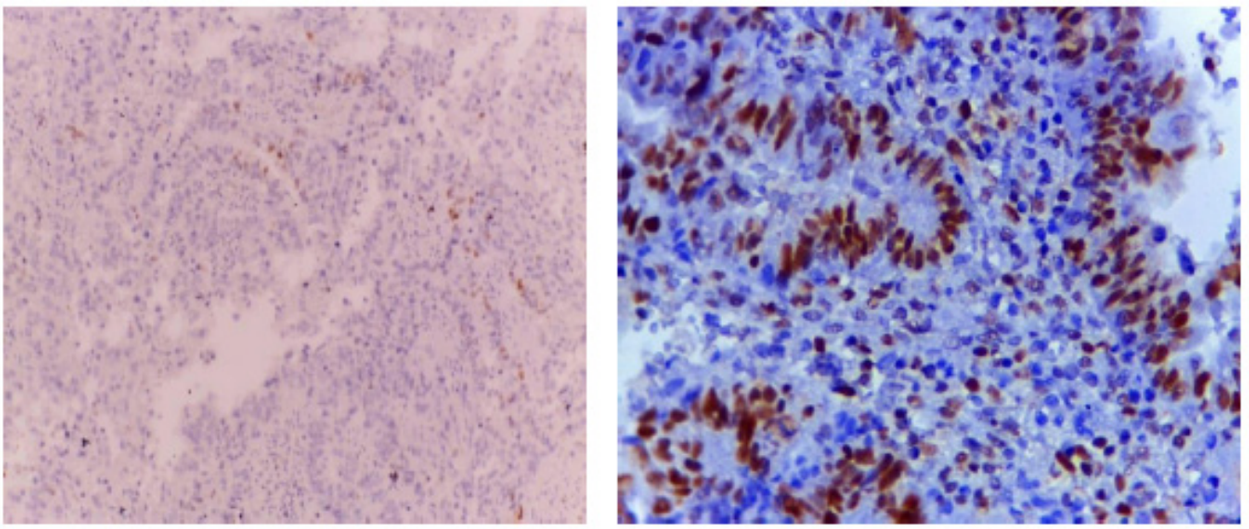
Patients were evaluated clinically, and radiologically according to the local guidelines for staging[15]. Myometrium, uterine cavity, cervix and pelvic lymph nodes were assessed during US staging to determine the extent of tumor[16,17]. CT with intravenous and oral contrast was performed to exclude parenchymatous metastasis, bowel involvement and pathological lymph nodes. Lymph nodes abnormality (size >1 cm in the shorter axis, rounded shape or necrosis) were marked to be suspicious for tumor involvement.

Pathological and immunohistochemical analysis

Tumor grade and histology were assessed preoperatively on D&C biopsy. All slides were read by two gynecological histopathologist to determine tumor type, grade, stage and the lymph vascular invasion according to the recent WHO Classification system 2020[18].

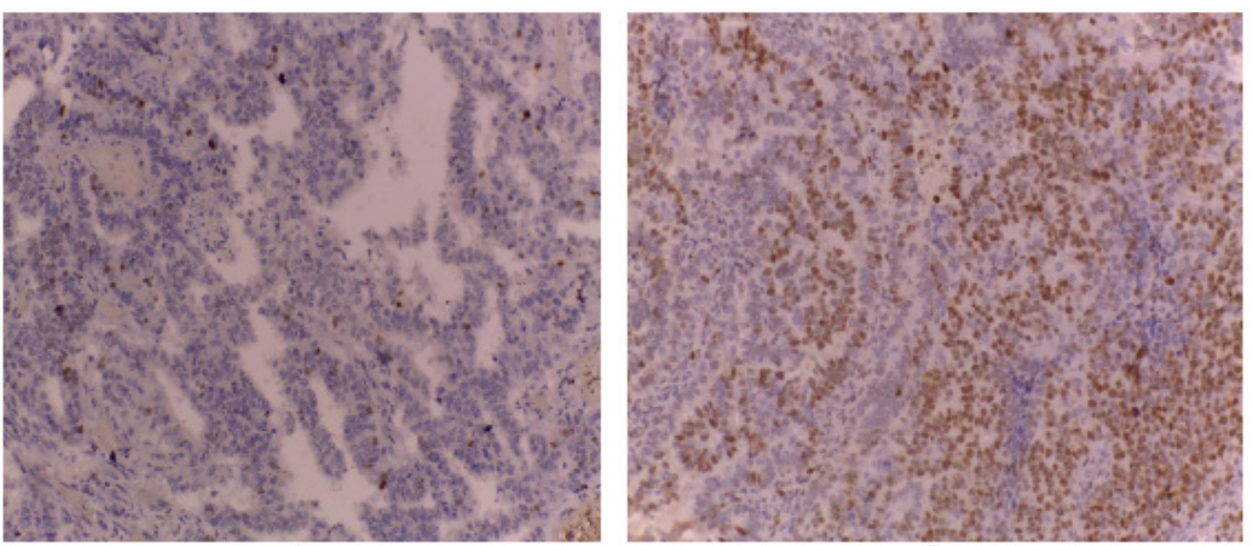
Immunohistochemical analysis was carried out on formalin-fixed and paraffin-embedded (FFPE) tissue sections for ER (Clone SP1, RBK 018-05, Zytomed, dilution 1:300), PR (Clone 16, product no. NCL-L-PGR-312, Novocastra, dilution 1:80), L1CAM (clone14.10, productno.826701, BioLegend, dilution1:100), and p53 (Clone DO-7, product no. M7001, DAKO, dilution 1:300).

Assessment of immunohistochemical staining (Figures 1, 2, 3, 4):



A B

Fig. 1: Representatve samples of ER Immunohistochemical expression in Endometrial carcinoma: (A) ER immunoreactivity in less than 10% of tumor cell nuclei (ABC X200). (B) ER immunoreactivity in more than 10% of tumor cell nuclei (ABC X400)



A B

Fig. 2: Representatve samples of PR Immunohistochemical expression in Endometrial carcinoma: (A) PR immunoreactivity in less than 10% of tumor cell nuclei (ABC X200). (B) PR immunoreactivity in more than 10% of tumor cell nuclei (ABC X200)

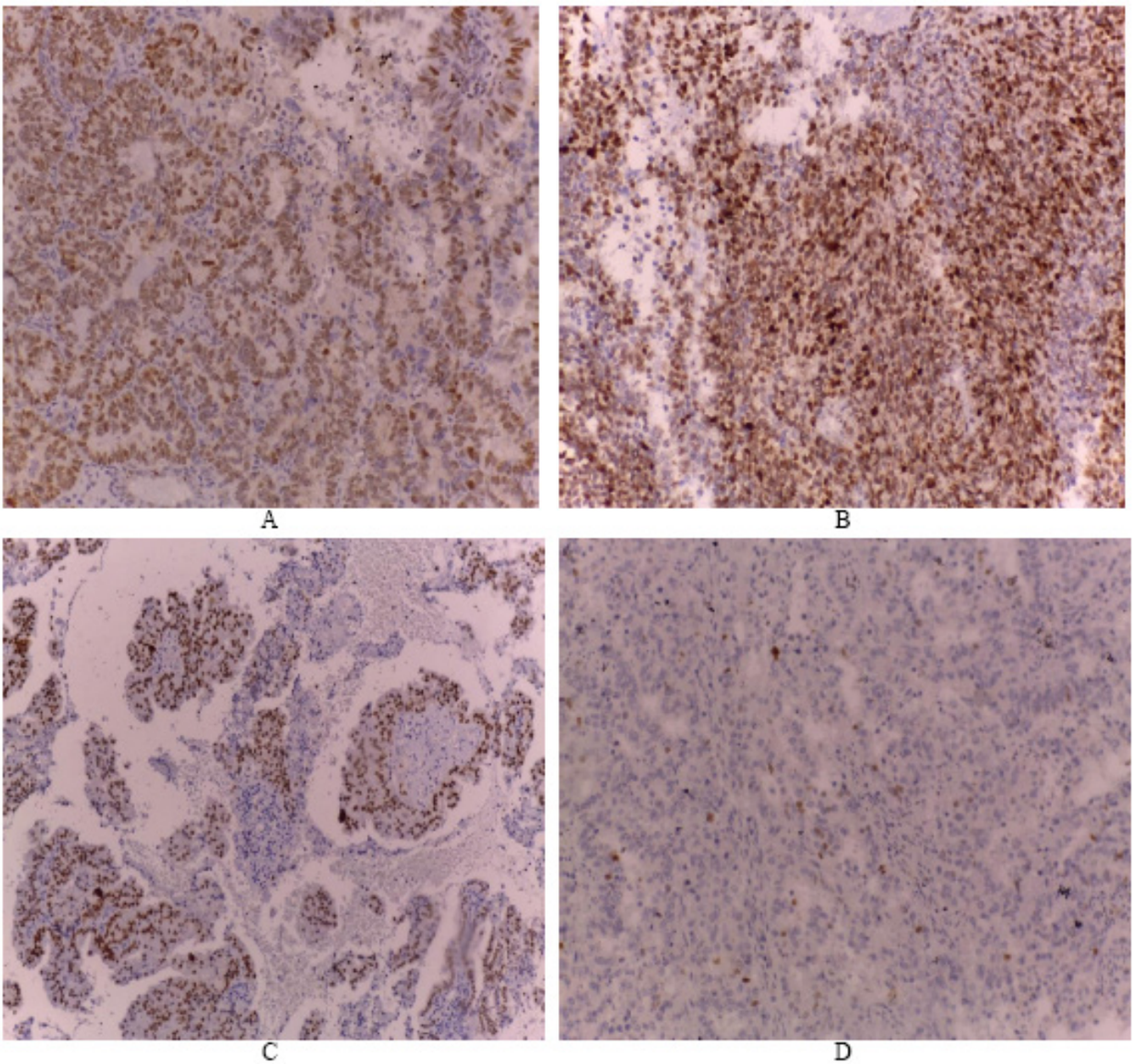


Fig. 3: Representatve samples of P53 Immunohistochemical expression in Endometrial carcinoma: (A) Positive P53 immunoreactivity >80% of tumor cell nuclei (ABC X200). (B Positive P53 immunoreactivity in >80% of tumor cell nuclei (ABC X200) (C) Positive P53 immunorpeactivity in non endometroid type in >80% of tumor cell nuclei (ABC X 100)&(D) P53 immunoreactivity in < 80% of tumor cell nuclei (ABC X400)

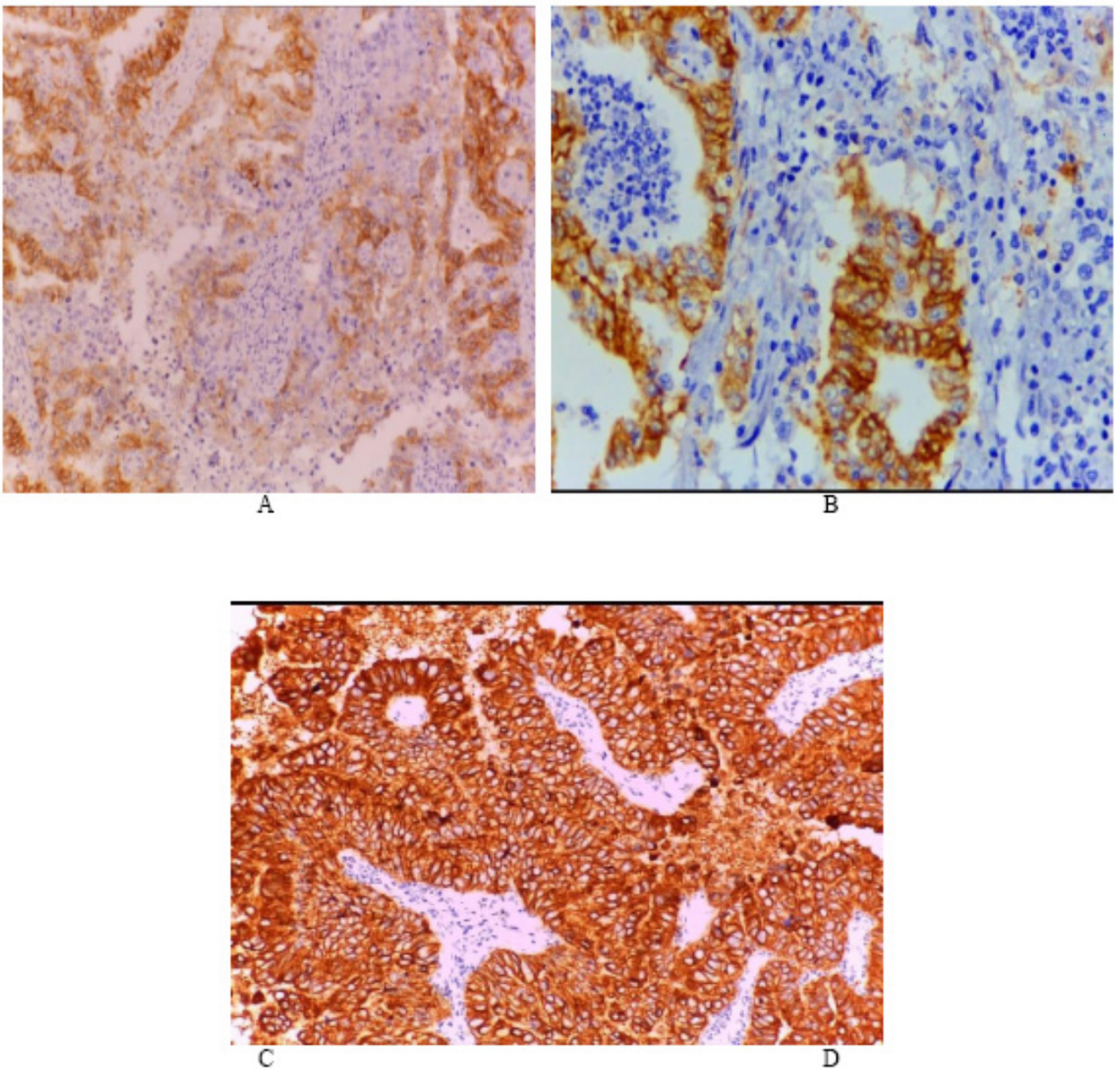


Fig. 4: Representatve samples of L1CAM, Immunohistochemical expression in endometrial carcinoma: (A) positive L1CAM, immunoreactivity (ABC X200). (B) positive L1CAM, immunoreactivity (ABC X400) (C) positive L1CAM, immunoreactivity non endometroid type (ABC X 200)

P53: It was considered as aberrant /abnormal (p53-abn) if > 80% of malignant cells revealed strong nuclear expression (over-expression) or if tumor cells completely lack nuclear expression (null-expression).

L1CAM: It was considered positive when tumor cells show distinct membranous staining in more than 10% of tumor cells (abnormal).

ER and PR: Were scored with number of stained tumor nuclei. Abnormal expression was considered if <10% nuclear staining was seen[19].

Post-operative ESMO-ESGO-ESTRO risk classification

According to data collected postoperatively including (Tumor grade, stage, histology, lymphovascular space invasion and myometrium invasion). Cases were classified into 5 groups: low, intermediate, high- intermediate, high and advanced/metastatic risk group[6].

Outcome measurement

We aimed to evaluate the prognosis of pre-operative

immunohistochemical expression of p53/L1CAM/ER/PR, to the ESMO-ESGO- ESTRO risk groups. Also, their added prognostic significance to lymph node status in EC.

Statistical analysis

Data were analyzed by NCSS 11 for windows (NCSS LCC., Kaysville, UT, USA) and IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were showed as the mean ± standard deviation (SD), while the qualitative data were assessed as percentage and frequency. We performed independent sample t test, Mann-Whitney test for not normally distributed data, chi-square and fisher exact for analysis of the qualitative data, Kaplan-Mayer test for analysis of survival Probability (*P-value*): *P-value* was significant if <0.05, highly significant if <0.001, and if >0.05; it was considered insignificant.

RESULTS

A total number of 96 patients were included. Thirty-three (33) cases were excluded due to lack of tumor tissue in tissue blocks, yielding 60 cases with a 36 months median follow-up.

Clinico-pathological data of the studied cases were reported. Median age distribution was 56.5 years (range 36-72 years). Pre-operative endometrial biopsy was EC endometrioid type (80%) and low-grade (I&II) EC (70%). Most patients were presented in early stage (stage I, II) 86.7%. Pre-operative immunohistochemical expression of ER/PR negative was 14 (23.3%), P53 abn was 11 (18.3%) and L1CAM+ in 8 (13.3%).

Twenty-nine patients (48.3%) had received adjuvant external beam radiotherapy (EBRT), including all patients of stage II, 15 patients of stage I and only 1 patient with stage III. Chemotherapy (taxol/carboplatin as the preferred regimen) was given to 8 patients as an adjuvant in stage III and as systemic treatment in stage IV patients.

Thirteen (21.7%) patients presented with recurrent EC and 8 (13.3%) patients died of whom 5 (62.5%) due to EC.

According to the ESMO-ESGO- ESTRO risk classification, cases were stratified as low risk in 31 cases & high risk in 18 cases; 51.7% & 30% respectively) as shown in (Table 1).

Table 1: Clinico-pathological data of the study group (N=60)

Patient characteristics	Median (range)
Age	56.5 (36-72)
	N (%)
Histology type	
Endometroid	48 (80)
Non-endometroid	12 (20)
Myometrial invasion	
<50 %	35 (58.3)
>50 %	25 (41.7)
Cervical involvement	20 (33.3)
LN involvement	
Positive	6(10)
Negative N0	23(38.3)
Unknown Nx	31(51.7)
LVSI	18 (30)
Grade	
I	15 (25)
II	27 (45)
III	18 (30)
FIGO stage	
I	39 (65)
II	13 (21.7)
III	6 (10)
VI	2 (3.3)
Risk groups	
Low	31(51.7)
Intermediate	5(8.3)
High-intermediate	4(6.7)
High	18(30)
Advanced/Metastatic	2(3.3)
Biomarker expression	
ER/PR negative	14 (23.3)
P53-abnormal	11 (18.3)
L1CAM positive	8 (13.3)
Adjuvant RTH	
VBT	
29229	5 (8.3)
EBRT	29 (48.3)
Chemotherapy	8 (13.3)
Outcome	
Relapse	13 (21.7)
Mortality	8 (13.3)

LN, lymph nodes; LVSI, Lymph-vascular space invasion; ER/PR, estrogen receptor/progesterone receptor; EBRT, external beam radiotherapy; FIGO, Federation International Gynecology Obstetric; L1CAM, L1 cell-adhesion molecule; VBT, vaginal beam(brachy) therapy.

Relation between immunohistochemical markers and tumor characteristics of the studied groups. There was statistically significant association between the

forementioned markers and histopathological findings (p<0.05), except histological type and grade had not significant correlation with abnormal P53 and L1CAM (Tables 2,3,4). Overall survival in relation to different immunohistochemical markers (Figures 5, 6, 7).

Table 2: Relation between ER\PR level and tumor characteristics of the studied groups.

N		Positive N=46		Negative N=14		*P value*
		%	N	%		
Histology Type	Endometroid	40	87.0	8	57.1	0.02 S
	Non	6	13.0	6	42.9	
Myometrial invasion	<50 %	35	76.1	0	0	<0.001 HS
	>50 %	11	23.9	14	100	
Cervical involvement		7	15.2	13	92.9	<0.001 HS
LN positive		2	4.3	4	28.6	0.02
LVSI		7	15.2	11	78.6	<0.001
Grade	I	15	32.6	0	0.0	0.03 S
	II	20	43.5	7	50.0	
	III	11	23.9	7	50.0	
Stage	I-II	44	95.7	8	57.1	<0.001 HS
	III-VI	2	4.3	6	42.9	
Risk group	Low	31	67.4	0	0.0	<0.001 HS
	Intermediate/ High intermediate	5	10.9	4	28.6	
	High/advanced	10	21.7	10	71.4	
Abnormal P53		0	0.0	11	78.6	<0.001
Positive L1CAM		1	2.2	7	50.0	<0.001
Chemotherapy		2	4.3	6	42.9	0.001 HS
Adjuvant RTH		15	32.6	14	100	<0.001
Relapse		4	8.7	9	64.3	<0.001
Mortality		3	6.5	5	35.7	0.01

Table 3: Relation between P53 and tumor characteristics of the studied cases.

N		Normal N=49		Abnormal N=11		*P value*
		%	N	%		
Histology Type	Endometroid	41	83.7	7	63.6	0.21
	Non	8	26.3	4	36.4	
Myometrial invasion	<50 %	35	71.4	0	0	<0.001
	>50 %	14	28.6	11	100	
Cervical involvement		9	18.4	11	100	<0.001
LN positive		2	4.1	4	36.4	0.008
LVSI		7	17.3	11	100	<0.001
Grade	I	15	30.6	0	0.0	0.09
	II	21	42.9	6	54.5	
	III	13	26.5	5	45.5	
Stage	I-II	47	95.9	5	45.5	<0.001
	III-VI	2	4.1	6	54.5	
Risk group	Low	31	63.3	0	0.0	0.001
	Intermediate/ High intermediate	6	12.2	3	27.3	
	High/advanced	12	24.5	8	72.7	
Negative ER/`PR		3	6.1	11	78.6	<0.001
Positive L1CAM		1	2	7	63.6	<0.001
Chemotherapy		2	4.1	6	54.5	0.001
Adjuvant RTH		18	36.7	11	100	<0.001
Relapse		4	8.2	9	81.8	<0.001
Mortality		3	6.1	5	45.5	0.004

Table 4: Relation between L1CAM and tumor characteristics of the studied cases.

N		Negative N=52		Positive N=8		*P value*
		%	N	%		
Histology Type	Endometroid	42	81.8	6	75.0	0.65
	Non	10	18.2	2	25.0	
Myometrial invasion	<50 %	35	67.3	0	0.0	<0.001
	>50 %	17	32.7	8	100	
Cervical involvement		12	23.1	8	100	<0.001
LN positive		2	3.8	4	50	0.008
LVSI		10	19.2	8	100	<0.001
Grade	I	15	28.8	0	0.0	0.21
	II	22	42.3	5	62.5	
	III	15	28.8	3	37.5	
Stage	I-II	50	96.2	2	25	<0.001
	III -VI	2	3.8	6	75	
Risk group	Low	31	59.7	0	0.0	0.007
	Intermediate/ High intermediate	6	11.5	3	37.5	
	High/advanced	15	28.8	5	62.5	
Negative ER/PR		7	13.5	7	87.5	<0.001
Abnormal P53		4	7.7	7	87.5	<0.001
Chemotherapy		2	3.8	6	75	0.001
Adjuvant RTH		21	40.4	8	100	0.002
Relapse		6	11.5	7	87.5	<0.001
Mortality		3	5.8	5	62.5	<0.001

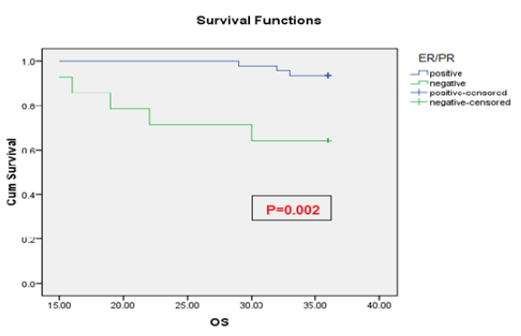


Fig. 5: Overall survival in relation to ER/PR expression

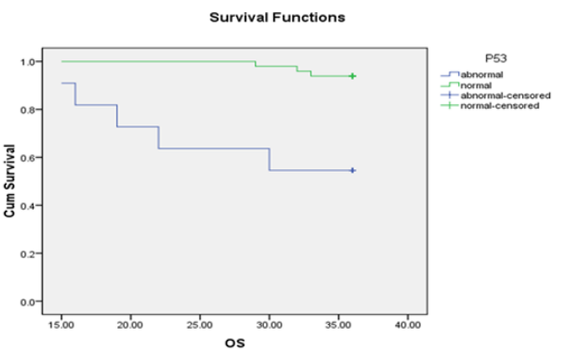


Fig. 6: Overall survival in relation to P53 expression

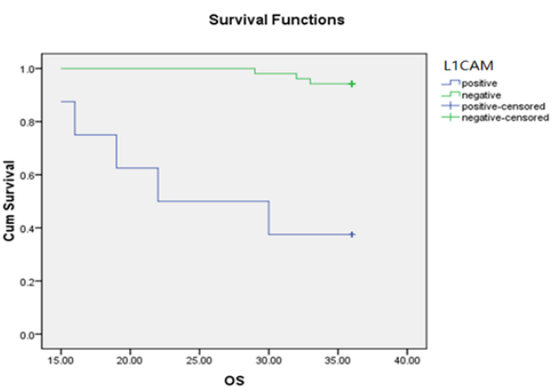


Fig. 7: Overall survival in relation to L1CAM expression

The three-year OS for patients with positive ER/PR expression was 93.5% while for patients with ER negative was 64.3% with highly significant difference (p =0.002).

Abnormal P53 expression associated with poorer OS (54.5%) than normal (93.9%) with significant difference (P <0.001). A highly significant difference was reported when we compared L1CAM expression in relation to OS with better OS with L1CAM –ve (94.2%) while L1CAM +ve (37.5%) (P <0.001). Progression free survival in relation to different immunohistochemical markers (Figure 8, 9, 10).

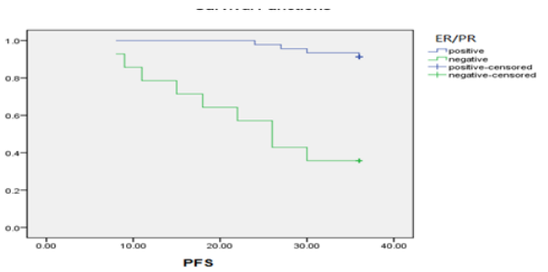


Fig. 8: Progression free survival in relation to ER/PR expression

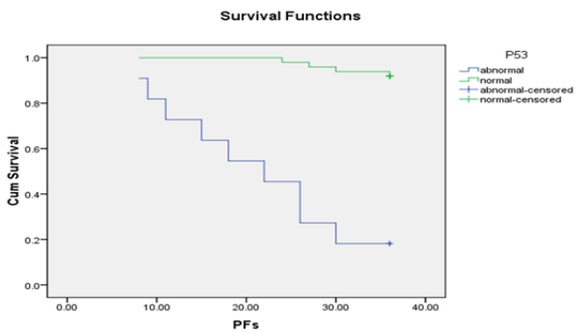


Fig. 9: Progression free survival in relation to P53 expression

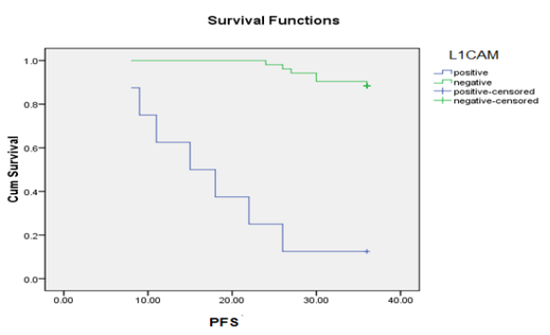


Fig. 10: Progression free survival in relation to L1CAM expression

Abnormal IHC markers (ER/PR–ve, abnormal P53 and L1CAM +ve) were associated with poorer PFS (35.7%, 18.2% and 12.5% respectively) with a high significant difference (P <0.001). Immunohistochemical expression in addition to ESMO-ESGO-ESTRO risk classification. Survival curves of the ESMO-ESGO-ESTRO risk groups in association to immune- expression are shown in (Figures 11,12,13).

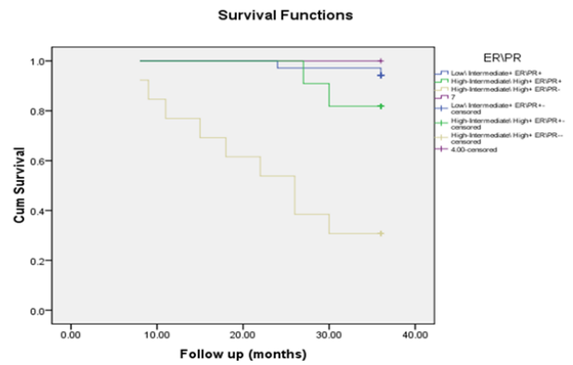


Fig. 11: PFS for ESMO-ESGO-ESTRO risk groups and ER/PR expression.

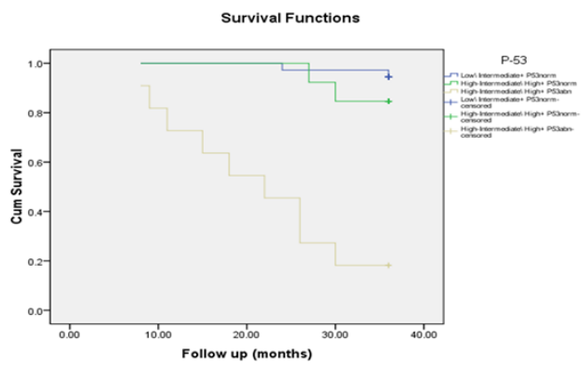


Fig. 12: PFS for ESMO-ESGO-ESTRO risk groups and P53 expression.

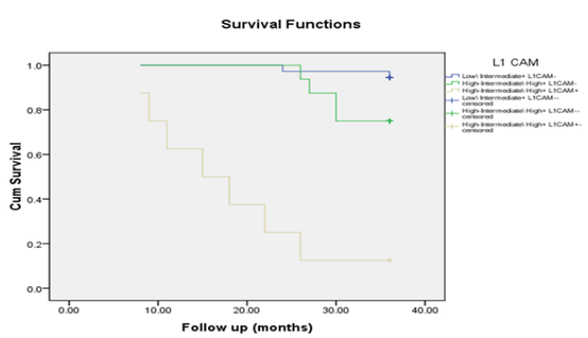


Fig. 13: PFS for ESMO-ESGO-ESTRO risk groups and L1CAM expression.

Patients showing abnormal immuno-expression (p53-abn, L1CAM+ or ER/PR-) and ESMO-ESGO-ESTRO risk group 'high intermediate and high and advanced/ metastatic' showed the lowest PFS compared with the other subgroups (P<0.001). Prognostic relevance of immunohistochemical expression in relation to the ESMO-ESGO-ESTRO risk classification. Multivariate analysis, showed that 'high intermediate and high and advanced/

metastatic' risk was independently linked to reduced PFS (HR 1.87 [CI 1.12-6.33] P= 0.002). L1CAM+ and P53-abn. were independently associated with reduced PFS (HR 2.23 [CI 2.34 -7.17] P= 0.002 and HR 1.12 [CI 0.943-3.21] P= 0.07) (Table 5).

Table 5: Univariate and multivariate Cox regression analysis of PFS.

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95% CI)	*P*
Low\Intermediate vs High-intermediate\High risk group	4.13 (1.22-8.51)	<0.001	1.87 (1.12-6.33)	0.002
P53 abnormal	2.87 (0.93-5.23)	0.004	1.12 (0.943-3.21)	0.07
ER\PR negative	2.45 (1.15-7.22)	0.01	2.11 (1.25-3.76)	0.987
L1CAM positive	3.77 (0.832-9.12)	<0.001	2.23 (2.34-7.17)	0.002

DISCUSSION

Determining the best surgical management and its adequate extent is important for treatment of patients recently diagnosed with EC with major difference between low and high-risk groups.

Several systems used preoperative data (histotype, grading & imaging) to determine patients' risk. According to these data, patients are categorized before the surgery into a risk group, accordingly a hysterectomy with salpingo-oophorectomy is decided, or extended management by para-aortic and pelvic lymphadenectomy. Stratification of a cases in a risk group before operations is important for management and overall survival and is a crucial question clinically.

In our study, 31 (51.7%) patients were of low-risk category and 18 (30%) were of high-risk category. Lymph node dissection was carried out in 29 patients (48.3%). However, if the risk stratification was previously assessed, only the high-risk group would go for lymph node dissection. Also, patients would go unnecessary adjuvant radiotherapy or repeated surgery due to inappropriate staging surgery, which could be easily overcomed by histopathological confirmation of tumor cells absence in lymph nodes. Surgical management with extensive lymph nodes dissection carry the hazards of post-operative morbidity without fulfilling oncological safety[20].

In this study, we aimed to evaluate the prognostic association of IHC biomarkers (L1CAM, ER, PR, and p53) preoperatively, to the ESMO-ESGO-ESTRO risk groups to determine if these markers could allow the segregation of cases into high- and low-risk groups to help the selection of the proper surgical extent.

Biologically, loss of PR occurs even before ER loss so eventually PR would be the best candidate regarding outcome. In the current study, ER/PR loss was evident in the 'advanced/metastatic 'risk group, with a possible link for spread, this was compatible with Karnezis *et al*.[21].

We found that patients with abnormal p53 were of 'high intermediate and high and advanced/metastatic' ESMO-ESGO-ESTRO risk group, similar findings were illustrated by Talhouk *et al*.[22].

L1CAM+ /p53-abn is a well-known prognostic factor in endometrial carcinoma as we illustrated[10,11,21]. However, in this study L1CAM+ positivity was slightly lower compared to other compared to other published data[10,23,24].

This may be attributed to the method of assessment which used small biopsies instead of tumor resections where focal expression of L1CAM was found /or at the invasive front predominantly[25]. L1CAM in our study was associated with advanced stage, lymphovascular invasion and high-risk group. Similar results were obtained by Van der Putten *et al*.[26] regarding advanced stage, lymphovascular space invasion.

In our study, abnormal IHC expression in the category of 'High-intermediate\High risk group, had the worst outcome (PFS). L1CAM+ve, ESMO-ESGO-ESTRO 'High-intermediate\High risk group and p53-abn, were independently associated with decreased PFS. Vrede *et al*. found similar findings regarding the outcome[19].

CONCLUSION

L1CAM and P53 could help in segregation of patients in the ESMO-ESGO-ESTRO risk classification to be used for refinement of LN directed surgery and selective adjuvant treatment.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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