The diagnostic and prognostic significance of L1 cell adhesion molecule, Sperm-associated antigen 9 and P53 expression in endometrial lesions (immunohistochemical study)

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Background
Endometrial carcinoma is the most common invasive neoplasm of the female reproductive tract. L1 cell adhesion molecule (L1CAM), Sperm-associated antigen 9 (SPAG9), and P53 have a role in the process of tumorigenesis and progression of several human malignant tumors, however, the role of them in cancer of endometrium is still not clear.

Aim
The study was performed to evaluate L1CAM, SPAG9, and P53 expression about different clinicopathological parameters in endometrial endometrioid adenocarcinoma.

Methods
The immunohistochemical study was performed on 50 cases of endometrial lesions including endometrial hyperplasia without atypia (10 cases), endometrial hyperplasia with atypia (10 cases), and endometrial endometrioid carcinoma (30 cases). Immunohistochemical staining techniques were used to evaluate the role of L1CAM, P53, and SPAG9 in endometrial endometrioid adenocarcinoma (EEC) and their relation to different clinicopathological data and patient's survival followed for 36 months.

Results
This study declared that both L1CAM and SPAG9 were found to be upregulated in EEC. Their over-expression was related to adverse clinicopathological parameters including high tumor grade, deep myometrial invasion, lymphovascular Invasion (LVI), and advanced tumor stage, while there was no significant relation between their expression and tumor size, cervical affection, and lymph node involvement. A high statistically significant link between L1CAM expression and poor patient survival was detected. Mutant type P53 was significantly related to adverse clinicopathological data as high tumor grade, deep myometrial invasion, lymphovascular space invasion (LVSI), and high tumor stage. There was a positive significant relation between mutant type P53 and high SPAG9.

Conclusions
The early identification of EEC in asymptomatic high-risk women may benefit from L1CAM and SPAG9 testing in combination with P53 protein. Also, they could be viewed as separate predictive variables in the EEC and might play a crucial part in the EEC's chemoresistance.

Keywords:
endometrial endometrioid adenocarcinoma, L1 cell adhesion molecule, P53, Sperm-associated antigen 9, survival

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Introduction

In the world, endometrial carcinoma (EC) is considered the sixth most frequent cancer in females. EC is predicted to cause 97 370 female fatalities and 417 367 new instances of diagnosis worldwide in 2020 (Sung *et al.*, 2021). The classification of ECs into type I and type II tumors is based on clinical and histological characteristics. About 85% of ECs are type I tumors, which are frequently detected in younger age groups and arise from an unusual hyperplastic precursor lesion. Since these tumors are frequently endometrioid endometrial carcinomas (EECs), are frequently well-differentiated, and frequently display little myometrial invasion, type I ECs frequently have a good prognosis (Colombo *et al.*, 2016).

A tiny portion of ECs are type II tumors which are typically discovered in elderly females, and usually form on top of an atrophic endometrium. Serous tumors, clear cell tumors, and perhaps grade 3 EECs are classified as type II tumors (Travaglino *et al.*, 2020).

Tumor type, stage, and degree of myometrial invasion and the presence of lymphovascular space invasion are entities that endometrial carcinoma (EC) patients are classified into four risk categories-low, intermediate, high-intermediate, and high—are identified (Siegel *et al.*, 2022).

L1 cell adhesion molecule (L1CAM) is a member of the superfamily of cell adhesion molecules known as immunoglobulins. It was originally discovered in the nervous system as it plays important roles in nervous system development, neuronal migration, neurite outgrowth on Schwann cells, and neurite myelination and fasciculation (Altevogt *et al.*, 2016).

L1CAM encourages cell migration, invasion, and metastasis in cancer cells. Many different forms of cancer, including colorectal, gastric, renal, breast, and melanoma, are linked to its expression and tumor progression (Altevogt *et al.*, 2016).

Sperm-associated antigen 9 (SPAG9) is a recently identified protein member of the cancer testis (CT) antigen family. It plays a functional role in the sperm-egg fusion and mitogen-activated protein kinase signaling pathway and plays a significant role in tumour development, cell growth, proliferation, and apoptosis by controlling the expression of genes involved in the epithelial-mesenchymal transition (Yu *et al.*, 2012).

Except for the testis, no other normal tissues express the special class of tumor antigens known as CT antigens. Moreover, immunological responses on both cellular and humoral levels to CT antigens have drawn attention to their potential in the early detection of cancer (Suri, 2006).

A potential biomarker for the early diagnosis of numerous human malignancies, including ovarian, cervical, and breast cancer, has recently been proposed as SPAG9. It is expressed in endometrial cancer tissue as well, but its clinical significance is unclear (Fédération Internationale de Gynécologie et d'Obstétrique [FIGO] Committee on Gynecologic Oncology, 2014). SPAG9 represents a promising candidate for cancer therapy owing to its expression pattern and immunogenicity in cancers of different origins (Suri *et al.*, 2012).

DNA repair, cell cycle arrest, and apoptosis are all functions of the tumor suppressor gene P53. As it is mutated in the early stages of lung, cutaneous, head and neck, and esophageal cancers, its overexpression is a characteristic of many human cancers (Lee *et al.*, 2018).

Patients and Methods

This current retrospective study was performed upon 50 cases of endometrial lesions divided as 10 cases of endometrial hyperplasia without atypia, 10 cases of endometrial hyperplasia with atypia and 30 cases of endometrioid endometrial carcinoma. The specimens of endometrial hyperplasia were taken by either curettage or hysterectomy, while in endometrioid adenocarcinoma specimens were taken by total hysterectomy with bilateral salpingo-oophrectomy with pelvic or both pelvic and paraaortic lymphadenectomy.

The cases of the current study consisted of archival formalin-fixed, paraffin embedded blocks processed during the period of 2018–2020 from the Pathology Department, faculty of medicine, Benha University.

The study was approved by the Research Ethics committee of Faculty of Medicine, Benha University, Egypt. No. (Rc 9-4-2023).

Histopathological study: Conventional hematoxylin and eosin (H and E) stain was performed on four-micron thick sections. Two of three experienced histopathologist examined all hematoxylin and eosin-stained slides and confirm diagnosis including histological subtype, stage, grade, and the presence or absence of lymphovascular invasion (LVI). The WHO Classification of Tumors of Female Reproductive Organs, 2020 was the frame which all specimens of cases were graded (FIGO Committee on Gynecologic Oncology, 2014).

The immunohistochemical study was performed by applying Avidin-Biotin complex technique and using antiL1CAM, anti P53and anti SPAG9 immunostains.

(1) Each primary polyclonal antibody was applied by adding one to two drops of either anti L1CAM antibody(clone 14.10, product no. 826701, BioLegend, dilution 1: 100), anti P53 (clone DO-7, product no. M7001, DAKO, dilution 1: 300)or anti SPAG9 with concentration (1: 150 dilution, ab12331; Abcam, Cambridge, UK) were applied to each section of them and slides were incubated at 4 C overnight.
(2) Chromogen diaminobenzine which is freshly prepared was used; it was applied to slides for 3–5 min then washed with the distilled water.
(3) Positive control: Staining of the nerves was used as internal positive control for L1CAM (Van der Putten *et al.*, 2016), human testis tissue for SPAG9

(Yan *et al.*, 2016), andnormal tonsil germinal centers B cells for P53 (Bodoor *et al.*, 2017).

(4) Negative control: Omitting of primary antibody during staining was used as negative control.

Immunohistochemical assessment

Evaluation and scoring of L1CAM immunohistochemically were performed by two pathologists for this study. A distinct membrane expression was considered positive. A case was considered a positive L1CAM expression if the slide showed over 10% of tumor cells were positive membrane staining (Van der Putten *et al.*, 2016).

Immunostaining of SPAG9 was scored by examination of representative tumor areas and evaluating the intensity and percentage of immunostained cells. A positive immunostaining was detected as cytoplasmic staining of the tumor cells. The intensity of SPAG9 cytoplasmic staining was also scored as 0 (no staining), 1 (weak), and 2 (marked). Percentage scores were assigned as 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The scores of each tumor sample were multiplied to give a final score of 0 to 8, as total score classified as less than 4 into negative and greater than or equal to 4 into positive or highly expressed (Yan *et al.*, 2016).

P53 was classified into wild type or mutant (excessive = strong diffuse overexpression in more than 90% of tumor cells or completely negative) phenotypes (Bodoor *et al.*, 2017).

Statistical analysis

Results were analyzed using SPSS (version 20). The Pearson correlation coefficient was used for statistical analysis. *P* value less than 0.05 was considered statistically significant and highly statistically significant when it was less than 0.01. Receiver operating characteristic (ROC) curve was also used to determine area under the curve, sensitivity and specificity of all markers, as area under the curve greater than 0.7, considered good. For assessment the statistical significance of the survival data the log-rank test was used. Survival data was plotted as Kaplan-Meier curves.

Results

Histopathological results

All clinicopathological data of endometrioid adenocarcinoma are shown in Table 1.

Table 1: Distribution of different clinicopathological data of endometrioid adenocarcinoma studied cases

Parameter	No. (%)
Age (mean±SD)=57.4 ± 9.47	
<50	24 (48%)
>=50	46 (52%)
Tumor size	
< 3.5 cm	26 (52%)
≥3.5 cm	24 (48%)
FIGO grade	
G1	19 (38%)
G2	18 (36%)
G3	13 (26%)
Depth of invasion	
No invasion	16 (32%)
< 50%	17 (34%)
≥50%	17 (34%)
LVI	
Negative	28 (56%)
Positive	22 (44%)
Cervical involvement	
Positive	24 (48%)
Negative	26 (52%)
Lymph node status	
Positive	16 (32%)
Negative	34 (68%)
FIGO stage	
I	19 (38%)
II	16 (32%)
III	15 (30%)

FIGO = Fédération Internationale de Gynécologie et d'Obstétrique, LVI = lymphovascular invasion

Immunohistochemical results

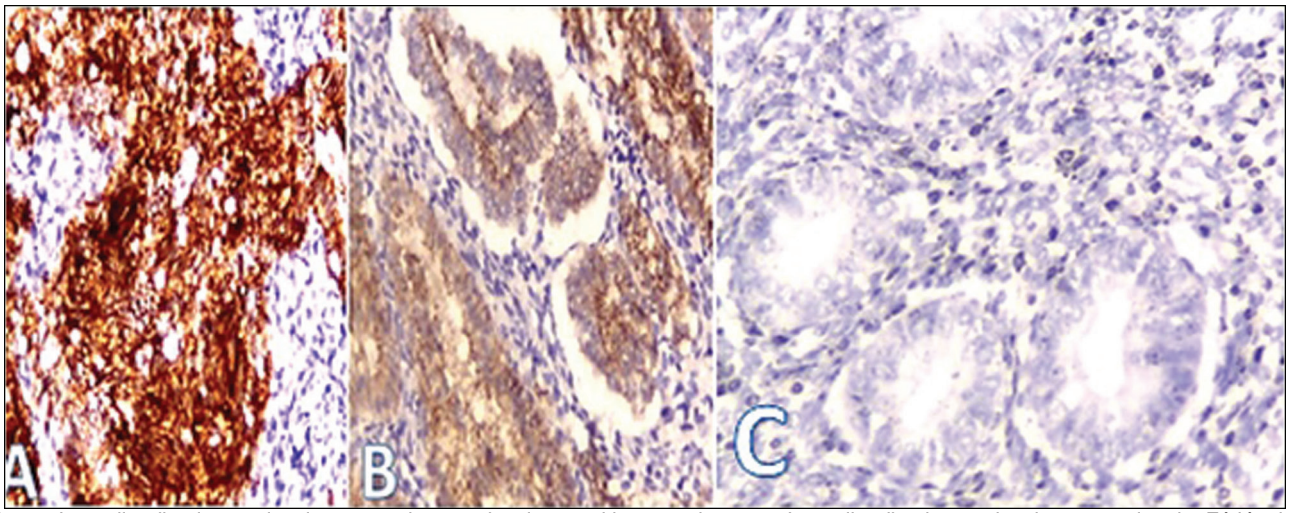
(1) Immunohistochemical expression of L1CAM and SPAG9 expression was significantly increased from endometrial hyperplasia without atypia to endometrial hyperplasia with atypia to endometrial endometrioid adenocarcinoma (EEC) (*P* value = 0.017 and 0.044, respectively), Fig. 1a.

(2) A significant statistical positive correlation was detected between L1CAM and SPAG9 expression and tumor grade (*P*-value= 0.046*, 0.016*), deep myometrial invasion (*P*-value= 0.008**, 0.001**), lympho-vascular invasion (*P*-value= 0.032*, 0.0325*) and The International Federation of Gynecology and Obstetrics (FIGO) stage (*P*-value= 0.026*, 0.001**). There was no significant correlation between its expression and tumor size, cervical involvement and lymph node involvement (*P*-value= 0.2, 0.8 and 0.2 and 0.13, 0.8, and 0.08, respectively) Table 2, Figs 1b, c, 2) respectively.

(3) There was a high statistically positive significant correlation between L1CAM and SPAG9 expression in the studied endometrial lesions (*P* value = 0.003**).

(4) Regarding P53, Mutant type P53 was significantly related to adverse clinicopathological data as high tumor grade (*P*-value= 0.011*), lymphovascular space invasion (*P*-value= 0.000**) and high tumor stage (*P*-value= 0.001**). Table 3, Fig. 3.

Figure 1



Shows L1 cell adhesion molecule expression: 1ashowing positive membranous L1 cell adhesion molecule expression in Fédération Internationale de Gynécologie et d'Obstétrique, grade III endometrial endometrioid adenocarcinoma (IHC, ABC X400),1b showing positive membranous L1 cell adhesion molecule expression in atypical endometrial hyperplasia (IHC, ABC X 400), 2c showing negative membranous L1 cell adhesion molecule expression in endometrial hyperplasia without atypia.

Table 2: Relation between different clinico pathological parameters and L1 cell adhesion molecule and Sperm-associated antigen 9 immunohistochemical expression in studied cases

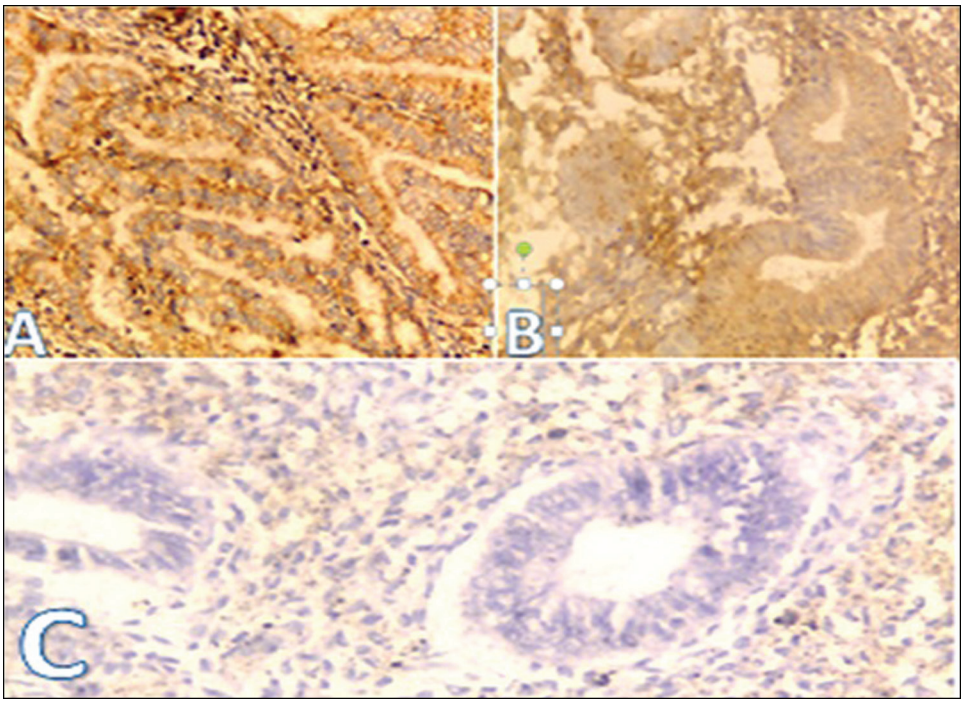
Clinico-pathological parameters	Total	L1 cell adhesion molecule expression		*P* value	Sperm-associated antigen 9 expression		*P* value
		Negative	Positive		Negative	Positive	
Tumor size							
<3.5 cm	16	7 (43.7%)	9 (56.25%)	0.2	9 (56.25%)	7 (43.75%)	0.13
≥3.5 cm	14	3 (21.4%)	11 (78.6%)		4 (28.5%)	10 (71.5%)	
FIGO Grade							
I	10	5 (50%)	5 (50%)	0.046*	7 (70%)	3 (30%)	0.016*
II	9	4 (44.4%)	5 (55.6%)		4 (44.4%)	5 (55.6%)	
III	11	2 (18.2%)	9 (81.8%)		2 (18.2%)	9 (81.8%)	
Depth of myometrial invasion							
No invasion	13	7 (53.8%)	6 (46.2%)	0.008**	9 (69.2%)	4 (30.8%)	0.001**
<50%	8	3 (37.5%)	5 (62.5%)		4 (50%)	4 (50%)	
≥50%	9	0	9 (100%)		0	9 (100%)	
LVI							
Negative	19	9 (47.4%)	10 (52.6%)	0.032*	11 (57.9%)	8 (42.1%)	0.035*
Positive	11	1 (18.2%)	10 (81.8%)		2 (18.2%)	9 (81.8%)	
Cervical invasion							
Negative	16	5 (31.24%)	11 (68.75%)	0.8	9 (56.25%)	7 (43.75%)	0.8
Positive	14	5 (35.7%)	9 (64.3%)		4 (28.6%)	10 (71.4%)	
Lymph node status							
Negative	23	9 (39.1%)	14 (60.9%)	0.2	12 (52.2%)	11 (47.8%)	0.08
Positive	7	1 (14.3%)	6 (85.3%)		1 (14.3%)	6 (85.3%)	
FIGO stage							
I	13	6 (46.2%)	7 (53.8%)	0.026*	10 (76.9%)	3 (23.1%)	0.001**
II	7	4 (57.1%)	3 (42.9%)		2 (28.6%)	5 (71.4%)	
III	10	0	10 (100%)		2 (20%)	8 (80%)	

FIGO = Fédération Internationale de Gynécologie et d'Obstétrique, LVI = lymphovascular invasion

(5) There was a positive significant relation between mutant type P53 and high SPAG9 expression (*P*-value= 0.015*), while no significant relation between L1CAM and mutant P53 (*P*-value= 0.12), Table 3.

(6) By using ROC analysis, L1CAM has sensitivity (70%) and specificity (55%) and SPAG9 has sensitivity (80%) and specificity (75%) in diagnosis of endometrioid adenocarcinoma cases (Table 4, Graph 1).

Figure 2



Shows SPAG9 expression:2a showing positive cytoplasmic SPAG9 expression in Fédération Internationale de Gynécologie et d'Obstétrique, grade II endometrial endometrioid adenocarcinoma (IHC, ABC X400), 2b showing positive cytoplasmic SPAG9 expression in endometrial hyperplasia with atypia (IHC, ABC X400), 2c showing negative cytoplasmic SPAG9 expression in endometrial hyperplasia without atypia.

(7) Kaplan-Meier analysis revealed a high statistically significant link between L1CAM expression and poor patient's survival (*P* value= 0.001), while no relation between patient's and SPAG9 expression was found (*P* value=0.784), (Table 5, Graph 2).

Discussion

Early detection of endometrial cancer, particularly its precancerous lesions, continues to be a crucial and developing problem in patient care and the effort to reduce endometrial cancer-related mortality (Chen *et al.*, 2022).

This study was held to determine the significance of L1CAM, SPAG9 and P53 expression in benign endometrial hyperplasia, atypical endometrial hyperplasia and EEC and association of these markers with different clinicopathological data.

The aberrant expression of L1CAM, which is directly linked to malignancy and therapy resistance as well as serving as a sign of poor prognosis, is found in a variety of tumor types as glioblastoma, high grade ovarian carcinomas and retinoblastoma (Bergmann *et al.*, 2010).

Concerning L1CAM immunohistochemical expression, it was found to increase significantly from endometrial precancerous lesions to EEC (*P* value= 0.017).

These results mean that L1CAM protein can be useful as a diagnostic tool in EEC

Also, L1CAM expression significantly increased in pancreatic ductal adenocarcinoma in relation to pancreas intraepithelial neoplasia (a precursor lesion for pancreatic adenocarcinoma), according to Bergman *et al.* (Bergmann *et al.*, 2010). A study by Chu *et al.* (Chu *et al.*, 2020), also concluded that serum L1CAM can be a possible biomarker for diagnosis of colorectal carcinoma. L1CAM diagnostic role can be explained that its expression is under control of Transforming growth factor-beta1 (TGF-b1) which is well known with its ability to regulate cell proliferation, growth, differentiation and cells movement in different human cancers. Also, it was implicated in upregulating integrin and fibro-nectin in malignant cells and activating the AKT and ERK pathways (Bergmann *et al.*, 2010).

A significant statistical positive correlation was detected between L1CAM expression and tumor grade (*P*-value= 0.046), deep myometrial invasion (*P*-value= 0.008), lympho-vascular invasion (*P*-value= 0.032) and FIGO stage (*P*-value= 0.026).

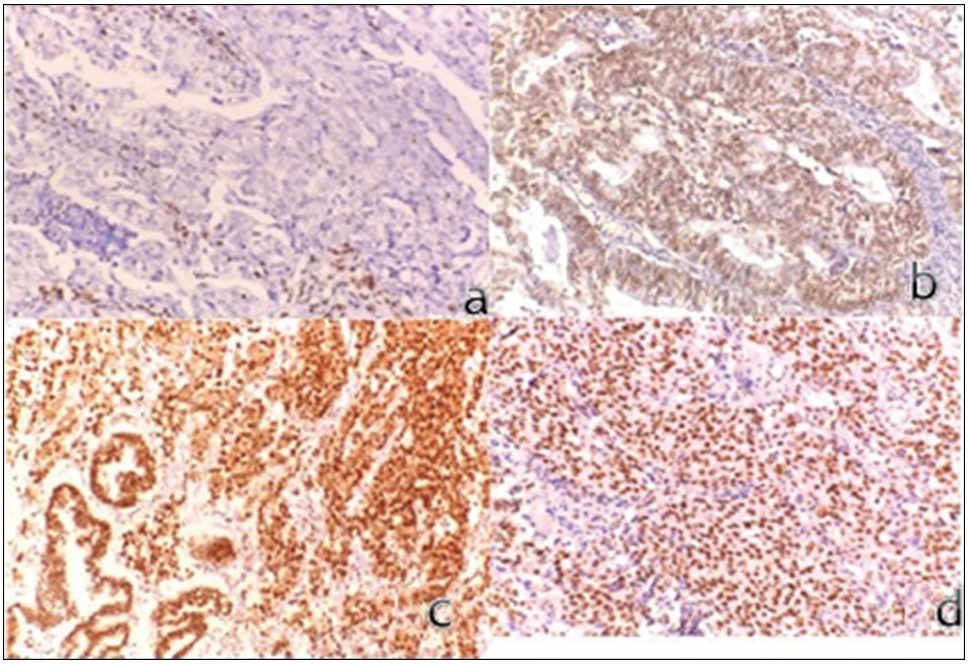
This was consistent with studies by Corrado *et al.* (Corrado *et al.*, 2018), and Elyamany *et al.* (Elyamany *et al.*, 2020). The current study showed no significant statistical correlation between its expression and tumor size, cervical involvement and lymph node

Table 3: Relation between P53 expression and different clinico pathological parameters and L1 cell adhesion molecule and Sperm-associated antigen 9 in studied endometrial endometrioid adenocarcinoma cases

Clinico-pathological parameters	Total	P53		*P* value
		Wild	Mutant	
Tumor size				
< 3.5 cm	16	9 (56.25%)	7 (43.75%)	0.6
≥3.5 cm	14	9 (64.3%)	5 (35.7%)	
FIGO Grade				
I	10	8 (80%)	2 (20%)	0.011*
II	9	6 (66.7%)	3 (33.3%)	
III	11	3 (27.3%)	8 (72.7%)	
Depth of invasion				
No invasion	13	9 (69.2%)	4 (30.8%)	0.26
< 50%	8	5 (62.5%)	3 (37.5%)	
≥50%	9	4 (44.4%)	5 (55.6%)	
LVI				
Negative	19	16 (84.2%)	3 (15.8%)	0.000**
Positive	11	2 (18.2%)	9 (81.8%)	
Cervical invasion				
Negative	16	12 (75%)	4 (25%)	0.077
Positive	14	6 (42.9%)	8 (57.1%)	
Lymph node status				
Negative	23	16 (69.6%)	7 (30.4%)	0.055
Positive	7	2 (28.6%)	5 (71.4%)	
FIGO stage				
I	13	11 (84.6%)	2 (15.4%)	0.001**
II	7	5 (71.4%)	2 (28.6%)	
III	10	2 (20%)	8 (80%)	
L1CAM expression				
Negative	10	8 (80%)	2 (20%)	0.12
Positive	20	10 (50%)	10 (50%)	
SPAG9 expression				
Negative	13	11 (84.6%)	2 (15.4%)	0.015*
Positive	17	7 (41.2%)	10 (58.8%)	

FIGO = Fédération Internationale de Gynécologie et d'Obstétrique, LVI = lymphovascular invasion
*significant, *P* value < 0.05, **highly significant, *P* value < 0.01

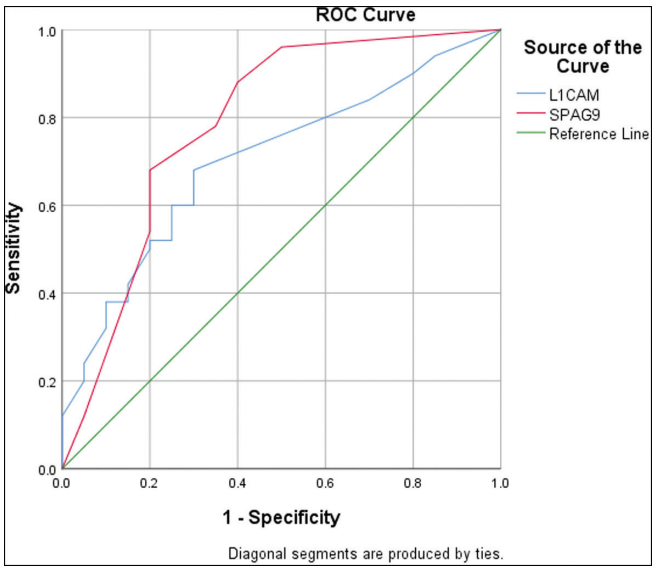
Figure 3



P53 expression:3a showing sparse nuclear P53 expression (Wild type pattern)in grade II endometrial endometrioid adenocarcinoma (IHC, ABC X200), 3b showing positive nuclear P53 expression (Mutant type pattern) in grade II endometrial endometrioid adenocarcinoma (IHC, ABC X200), 3c showing positive nuclear P53 expression (Mutant type pattern) in grade III endometrial endometrioid adenocarcinoma (IHC, ABC X200), 3d showing positive nuclear P53 expression (Mutant type pattern)in grade III endometrial endometrioid adenocarcinoma (IHC, ABC X400).

involvement in endometrioid adenocarcinoma (*P*-value= 0.2, 0.8, and 0.2, respectively). This was against a study by Elyamany *et al.* (Elyamany *et al.*, 2020), This disparity may be owed to different histological subtypes used in this study. The invasive activity of L1CAM can be owed to upregulation of expression of metalloproteinase-2 (MMP-2) and MMP-9 through a Tyrosine Aminotransferase 3 activation (Elyamany *et al.*, 2020).

Graph 1



Validity of screening test to predict cut off points in different markers.

Sperm-associated antigen 9 (SPAG9) was found to be over expressed in EEC in relation to precancerous lesions in the form of endometrial hyperplasia with or without atypical features (*P* value= 0.044). This means that SPAG9 can be used as a novel diagnostic tool in EEC. These results were in agreement with Baser *et al.* (Baser *et al.*, 2013), Studies by Ramadan *et al.* (Ramadan *et al.*, 2022), and Xiao *et al.* (Xiao *et al.*, 2019a), revealed upregulated SPAG9 expression in colorectal carcinoma, prostatic adenocarcinoma and hepatocellular carcinoma compared with adjacent non tumorous tissues by immunohistochemical, western blot and cell culture techniques. Its diagnostic role can be owed to that SPAG9 upregulation can activate JNK signaling pathway to play its oncogenic effect and promote tumor cell proliferation (Xiao *et al.*, 2019a).

Table 4: Validity of screening test to predict cut off points in different markers

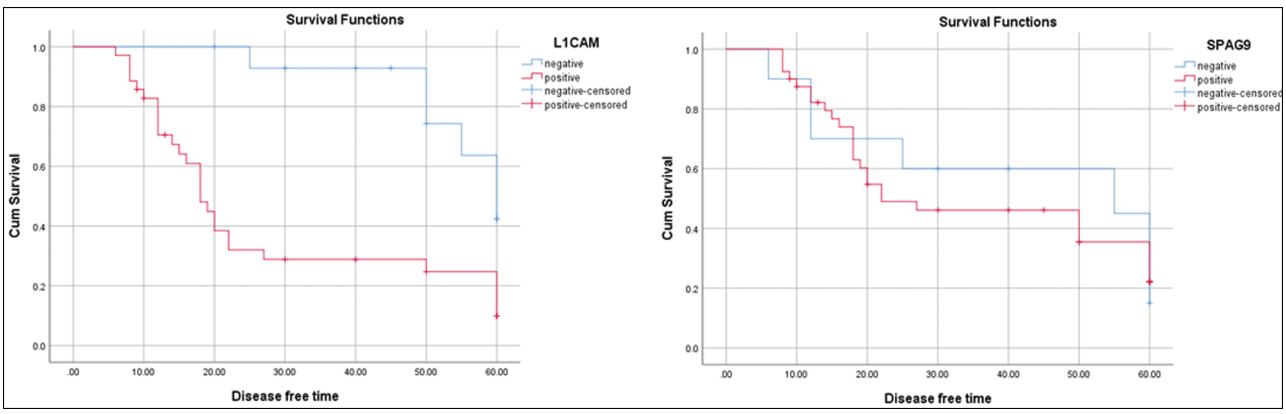
Test Result Variable(s)	Area under the curve	*P* value	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
SPAG-9	0.786	0.001	80.0	75.0	88.9	60.0	73.3
L1CAM	0.703	0.008	70.0	55.0	79.5	42.3	61.3

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

Table 5: Relation between L1 cell adhesion molecule and Sperm-associated antigen 9 expression and disease-free survival

L1 cell adhesion molecule	Mean survival	95% C.I	Log Rank test	*P* value
Positive	27.688	20.52–34.85	11.893	0.001
Negative	55.112	49.68–60.00		
SPAG9	Mean survival	95% C.I	Log Rank test	*P* value
Positive	35.183	28.14–42.23	0.075	0.784
Negative	40.750	25.64–55.86		

Graph 2



Relation between L1 cell adhesion molecule and Sperm-associated antigen 9 expression and disease-free survival.

It was found that high SPAG9 expression had a significant statistical correlation with high grade of tumor (*P* value = 0.016), deep myometrial invasion (*P* value = 0.001), presence of lymphovascular space invasion (*P* value = 0.035) and advanced tumor stage (*P* value = 0.001). These results came in line to results by Zhao *et al.* (Zhao *et al.*, 2016), in the contrary, SPAG9 pathway analysis by Gullo *et al.* (Gullo *et al.*, 2023), produced findings that it was substantially linked to low grade and better prognosis. Different numbers of cases examined, variations in techniques can all be used to explain this disparity.

There was no significant relation between SPAG9 immunohistochemical expression and tumor size, cervical affection and lymph node involvement. These outcomes against with those of Baser *et al.* (Baser *et al.*, 2013).

Previous studies found that SPAG9 was significantly related to astrocytoma tumor grade, Gleason grade in prostatic adenocarcinoma and tumor grade in breast cancer (Sinha *et al.*, 2013). This can be its ability to activate matrix metalloproteinases, thus affecting cancer cell proliferation, invasion, and chemoresistance (Sinha *et al.*, 2013). Also, study by Xiao *et al.* (Xiao *et al.*, 2019b), revealed that SPAG9 over expression can upregulate Cyclin D1 and CDK2 protein expression and accelerate cell cycle and this is responsible for tumor cell invasive power.

There was a high statistically positive significant correlation between L1CAM and SPAG9 expression in the studied endometrial lesions (*P* value = 0.003). No similar previous studies stated the relation between both markers in EEC, however Piastra *et al.* (Piastra *et al.*, 2022), found silencing of both L1CAM and SPAG9 mRNA can help in treatment of melanoma in animal models. This can be explained by that over expression of SPAG9 as well as L1CAM was associated with upregulation EMT (Epithelial Mesenchymal Transition) related genes including Snail, Slug, Ncad, Ecad, and Twist and EMT related genes had been demonstrated in endometrioid adenocarcinoma cell lines (Piastra *et al.*, 2022).

In the early identification of EEC cases, ROC analysis has shown that SPAG9 is more sensitive and specific than L1CAM.

There was a positive significant relation between mutant type P53 and high SPAG9 expression (*P*-value= 0.015), while no significant relation between high L1CAM and mutant P53 (*P*-value= 0.15).

Study of Huang *et al.* (Huang *et al.*, 2023), revealed that high SPAG9 mRNA and protein expression was associated with mutant P53 in prostatic adenocarcinoma.

As revealed by using Kaplan Meier analysis over expression of L1CAMwas found to be related to poor prognosis and short patient's survival (*P* value = 0.001).

This was in agreement of results by Romani *et al.* (Romani *et al.*, 2022), These findings can be explained by its ability to modulate several intracellular signaling pathways as PI3K/Akt and MAPK pathways, affecting proliferation and apoptosis.

Therefore, the relationship between L1CAM and cancer stemness in colorectal cancer has been demonstrated by Ganesh *et al.* (Ganesh *et al.*, 2020). where L1CAM identifies a population of stem-like cells that express CD133/CD44 and are endowed with increased chemoresistance and tumorigenic potential (Ganesh *et al.*, 2020). Additionally, L1CAM and CD133 define a population of ovarian cancer stem cells (CSCs), which exhibit improved radio resistance, improved clonogenic properties, self-renewal capability, and superior tumor growth in nude mice (Terraneo *et al.*, 2020).

Conclusions

The early identification of EEC in asymptomatic high-risk women may benefit from L1CAM and SPAG9 testing besides P53 testing. Also, they could be viewed as separate predictive variables in EEC and might play a crucial part in the EEC's chemotherapy by therapeutic inhibition of them. To confirm their function, additional researches utilizing a larger number of cases and extensive in vivo and in vitro studies.

Author Contributions

All authors shared equally in collection and tabulation of the data for this article.

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Conflicts of interest

Conflicts of Interest of each author/contributor: the authors declare that they have no competing interest

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