Immunohistochemical Expression of Epithelial Cell Adhesion Molecule (EpCAM) in Epithelial Ovarian Carcinoma

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Abstract

Background: Ovarian carcinoma is the 3rd highest frequent cancer of female reproductive system and the 5th most prevalent leading reason of death from cancer in females globally. Epithelial cell adhesion molecule (EpCAM) is a type I transmembrane protein that is overexpressed in several neoplasms. Recently, it has been regarded as an emerging indicator for cancer stem cells (CSCs) across several types of neoplasms. Recent studies indicate that EpCAM may be a potential key player in initiation, growth, and metastatic spread of tumors.

Objectives: This research was conducted to assess the expression levels of EpCAM in epithelial ovarian carcinoma (EOC) and analyze its association with established clinicopathological factors.

Materials and methods: Fifty tissue samples of EOC preserved in formalin and embedded in paraffin were examined for EpCAM expression using immunohistochemistry (IHC). EpCAM expression was statistically analyzed for its association with various clinicopathological variables.

Results: EpCAM expression in EOC was frequently upregulated in large, poorly differentiated, and advanced tumors and it showed a strong relationship with tumor laterality, the presence of peritoneal deposits and lymphovascular invasion (LVI).

Conclusion: EpCAM molecule has an important role in carcinogenesis; it could promote the progression, invasion, and metastasis of EOC. It could act as a potential predictive biological marker and therapeutic target.

Keywords: Ovarian carcinoma; EOC; EpCAM; Cell adhesion; CSCs.

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Introduction

Ovarian carcinoma ranks as the 3rd most frequent gynecological malignant tumor and the 5th leading cause of cancer deaths among women worldwide **(Sung et al., 2021)**. Ovarian cancer is a diverse malignancy made up of various histological subtypes. Malignant epithelial tumors are the most frequent histological type, accounting for about 90% of ovarian cancers **(Kossaï et al., 2018)**. It has the least favorable prognosis and the highest mortality rate among other gynecological malignant tumors. **(Momenimovahed et al., 2019)**.

Based on the 4th edition (2014) of the World Health Organization (WHO) classification system of tumors of the female genital system, EOC is divided into several histologic subtypes with different pathogenesis and prognosis, such as: serous, mucinous, endometrioid, clear-cell, transitional-cell, and undifferentiated carcinomas **(Kurman et al., 2014)**.

However, the 5th edition of the WHO classification (2020) divides ovarian carcinomas into five main types according to histopathological features, immune profile, and molecular profile: high-grade serous carcinoma (70%), endometrioid carcinoma (10%), clear cell carcinoma (6-10%), low-grade serous carcinoma (5%), and mucinous carcinoma (3-4%). The combination of modern diagnostic criteria with immune and molecular profiles improves the diagnostic and prognostic knowledge of these different histological subtypes **(Holger, 2020; De Leo et al., 2021).**

Invasion and metastasis are the main causes of high mortality rates in individuals diagnosed as ovarian carcinoma. The invasive properties of ovarian malignancy are regulated by the alteration of cell adhesion molecules (CAMs) with further upregulation of matrix metalloproteinases (MMPs) **(Laszlo et al., 2018)**.

Furthermore, poor survival rates are primarily attributed to relapse and chemoresistance, which may in part be caused by the presence of CSCs. They are believed to be key players in metastasis and recurrence. Previous studies have shown that CSCs are more resistant to conventional chemotherapeutic agents than non-CSCs **(Kenda and Klun, 2019).**

EOC exhibits aggressive biological behavior; the detection of metastasis-related factors and molecular alterations associated with different stages of EOC progression has been a major challenge to research for several decades. Therefore, there is an increasing critical need to identify new molecular biomarkers that can be used as prognostic markers to predict the clinical outcome and help in the development of more effective and targeted therapeutic agents (**Atallah et al., 2021)**.

EpCAM, also known as cluster of differentiation 326 (CD326), is classified as type I membrane-pound glycoprotein. It is encoded by the GA733-2 gene found on chromosome 2 (location 2p21). It is primarily reported as one of the CAMs that mediates cell-cell adhesions within the majority of normal epithelial tissues. EpCAM has been recognized as a CSCs marker (**Schnell et al., 2013)**. EpCAM serves as a biomarker for diagnosis and prognosis for different neoplasms. It has been demonstrated to play a significant role in the process of carcinogenesis, but its precise biological functions in the initiation and progression of tumors remain unclear. Most epithelial tumors exhibit EpCAM reactivity with variable expression in different histologic types; this variation might be of significant prognostic value (**Liu et al., 2022).**

Few previous studies have been conducted to evaluate EpCAM expression in ovarian carcinoma in relation to the clinical outcome with controversial results. This research aimed to assess EpCAM expression in EOC and link it to various clinicopathological factors to better define its prognostic significance.

Materials and methods

Tissue samples*:* This is a retrospective observational study. Fifty tissue blocks preserved in formalin and embedded in paraffin of EOC specimens were enrolled from the Pathology Laboratory, Sohag University Hospitals and Sohag Oncology Center during the time period from June 2017 to June 2022.

Hematoxylin and eosin (H&E) stained sections were reviewed again to verify diagnosis and determine tumor grade. Histological classification of EOC was done according to the 5th edition of WHO classification **(WHO, 2020).** Staging was carried out based on the International Federation of Gynecology and Obstetrics classification system (FIGO) **(Berek et al., 2018).** The clinical and surgical data were obtained from the patient's medical documents.

Inclusion criteria: Specimens of EOC that had all required clinical information.

Exclusion criteria*:* Insufficient clinical data or cases with a history of pre-operative anti-cancer therapy

Ethical considerations: The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Approval was generated by the Committee of Medical Ethics at the Faculty of Medicine, Sohag University (Registration number: Soh-Med-22-09-15) and enrolled in the ClinicalTrials.gov Protocol Registration System (PRS) under the ID: NCT05576519 on **12/10/2022**.

Immunohistochemical detection of EPCAM

Immunohistochemical (IHC) EpCAM staining was done using avidin-biotin-peroxidase complex method. The formalin-fixed paraffin-embedded tissue blocks were sectioned at 4 µm thickness, mounted on coated slides, deparaffinized in xylene, and rehydrated in decreasing concentrations of alcohol. To block endogenous peroxidase, tissue samples were immersed in 0.3% hydrogen peroxide for 20 minutes. Antigen retrieval was accomplished by boiling tissue samples in 0.01 mmol/L sodium citrate buffer (pH 6), in a microwave oven at 750 Watt for 15 minutes, divided into 3 cycles of 5 minutes each. Mouse monoclonal anti-EpCAM antibody (dilution 1/100, Catalog # 94538, Clone VU-1D9, THERMO SCIENTIFIC Corporation, Fremont, USA) was incubated with tissue sections overnight at 4ºC. The next day, tissue sections were treated with a biotin-labeled goat secondary antibody at room temperature for 30 minutes. Then, streptavidin peroxidase was applied to the tissue at ambient temperature for 10 minutes. Tissue sections were exposed to freshly prepared diaminobenzidine (DAB) chromogen by adding DAB chromogen to DAB substrate at a concentration of 1:25. Between incubations, tissue sections were washed in PBS. Finally, tissue sections were dehydrated, cleared, and mounted as usual after being counterstained with Mayer's hematoxylin.

Positive and Negative Controls

Breast carcinoma sections known to be positive for EpCAM were used as positive controls for the IHC process as recommended in the data sheets. Negative control sections were

prepared from EOC, but by using PBS in place of the primary antibody.

Assessment of of EpCAM expression

Corresponding H&E sections were examined side by side with immune-stained sections. EpCAM positivity was identified as a brownish membranous and/or cytoplasmic staining of neoplastic cells, ignoring the stromal staining.

Scoring of immunoreactions

EpCAM expression was assessed independently by two pathologists who were blinded to clinical or pathological information. EpCAM expression was measured following the method described by **Tayama et al. (2017)** through computation of a total immunoreactive score (IRS), which was determined by multiplying the proportion of immunoreactive cells (quantity score) with the staining intensity (intensity score). Staining amount was graded as follows; (0: none, 1: 1-25%, 2: 26-50%, 3: 51-75%, and 4: >75%). The staining intensity was rated on a 0-3 scale (0: no staining, 1: weak, 2: moderate, and 3: strong). The overall score varied between 0 and 12. A score 0-4 was defined as low expression, while a score ≥ 6 was defined as high expression **(Tayama et al., 2017).**

Statistical analysis

To compare categorical variables, statistical evaluation was conducted using either Chi-square test or Fisher's exact test. P value less than 0.05 was referred to as significant. For qualitative data, frequencies and percentages were used, whereas quantitative data were expressed as mean± standard deviation (SD), median, and range. Statistical Package for the Social Sciences (SPSS) software version 20 was utilized for statistical analysis.

Results

This study included 50 cases of malignant surface epithelial tumors of the ovary. The patients' age mean ±SD was 53.12±8.84 and a median of 53 years (range, 36-72). Tumor size was ranged from 7-22 cm, with a mean ±SD and median of 11.82± 3.72 cm and 12 cm, respectively. Histologically, the tumors were classified as high grade serous, low grade serous, mucinous, endometroid, and transitional cell carcinoma in 20, 9, 9, 8, and 4 cases, respectively. Among the investigated cases, 23 (46%) of the tumors were low grade, while 27 (54%) were high grade. The clinicopathological findings of the included cases were summarized in **(Table.1).**

Table 1. Clinicopathological characteristics in studied EOC Cases

Parameters	**Total Number** (50)	**Percentage**
Age		
<50	21	42%
≥50	29	58%
Histological variant		
High grade Serous carcinoma	20	40%
Low grade serous carcinoma	9	18%
Mucinous carcinoma	9	18%
Endometroid carcinoma	8	16%
Transitional cell carcinoma	4	8%
Tumor size (cm)		
<10	19	38%
≥10	31	62%

Laterality		
Unilateral	22	44%
Bilateral	28	56%
Grade		
Low	23	46%
High	27	54%
FIGO staging		
I	5	10%
II	15	30%
III	24	48%
IV	6	12%
Peritoneal deposits		
Absent	23	46%
Present	27	54%
LVI		
Negative	24	48%
Positive	26	52%
Necrosis		
Absent	19	38%
Present	31	62%

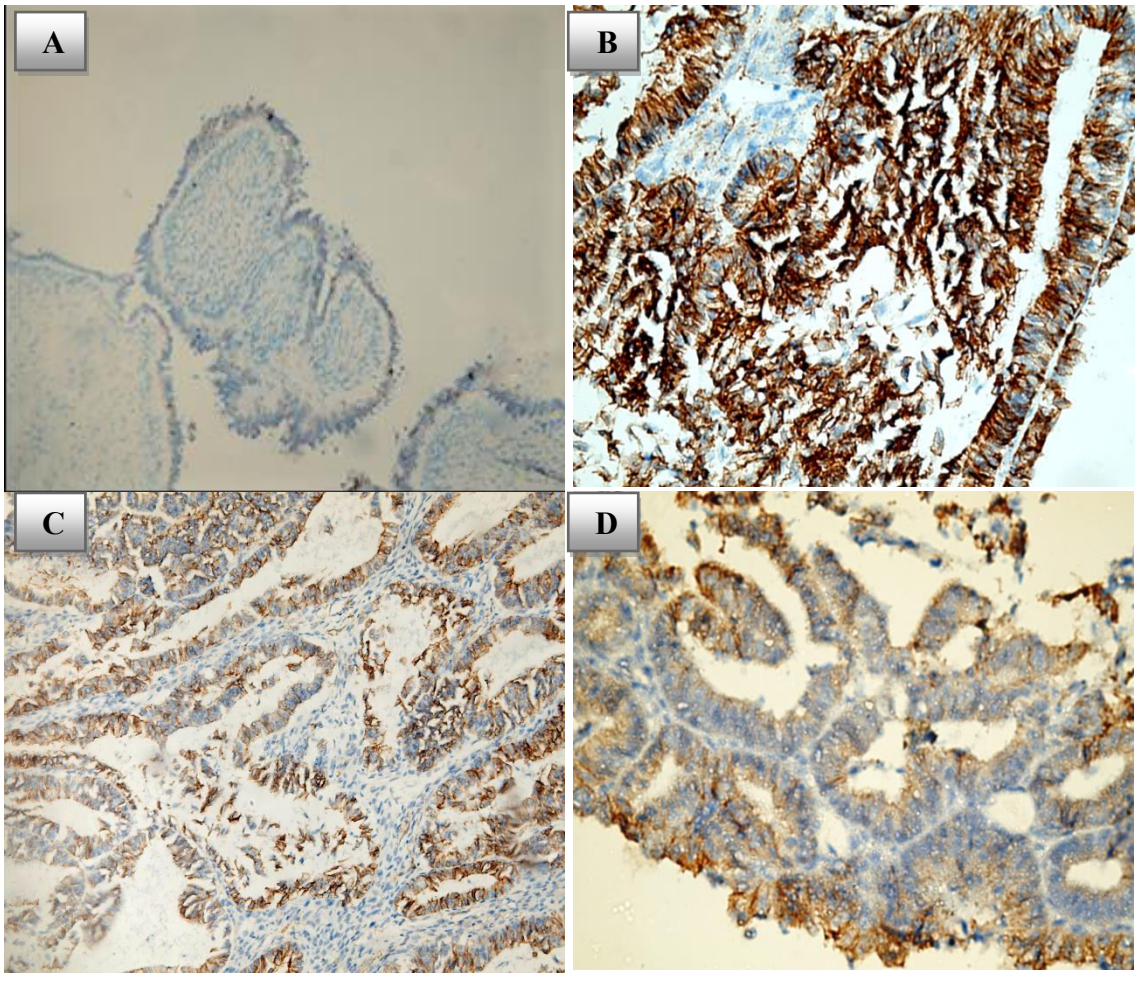
High EpCAM expression was observed within tumor cells in 28 (56%) of cases, while 22 (44%) of cases revealed low expression. The expression was mainly membranous **(Fig.1)**. From a statistical perspective, elevated EpCAM expression showed a positive association with larger tumor size (p= 0.033), less differentiated tumors (p= 0.027), and advanced stages (p=0.007). This study additionally found a significant positive association between EpCAM overexpression and the existence of peritoneal deposits and LVI (p=0.005 and p=0.002, respectively). Conversely, statistical evaluation of EpCAM expression in relation to patients' age, histologic subtype, and presence of necrosis showed no statistical significance, as shown in **(Table.2).**

Table 2. Statistical Correlations between EpCAM Expression and Clinicopathological Parameters in EOC Cases

Parameters	**NO of cases**	**EpCAM expression**		**P value**
	50	**Low N=22(44%)**	**High N=28(56%)**	
Age				
<50	21(42%)	12(57.1%)	9(42.9%)	0.111
≥50	29(58%)	10(34.5%)	19(65.5%)	
Histological variant				
High grade Serous	20(40%)	5(25%)	15(75%)	0.058
Low grade serous	9(18%)	4(44.4%)	5(55.6%)	
Mucinous	9(18%)	4(44.4%)	5(55.6%)	
Endometroid	8(16%)	7(87.5%)	1(12.5%)	
Transitional cell carcinoma	4(8%)	2(50%)	2(50%)	
Tumor size (cm)				
<10	19(38%)	12(63.2%)	7(36.8%)	0.033

≥10	31(62%)	10(32.3%)	21(67.7%)	
Laterality				
Unilateral	22(44%)	14(63.6%)	8(36.4%)	0.013
Bilateral	28(56%)	8(28.6%)	20(71.4%)	
Grade				
Low	23(46%)	14(60.9%)	9(39.1)	0.027
High	27(54%)	8(29.6%)	19(70.4%)	
FIGO staging				
I	5 (10%)	5(100%)	0	0.007
II	15(30%)	8(53.3%)	7 (46.7%)	
III	24(48%)	9(37.5%)	15(62.5%)	
IV	6 (12%)	0	6 (100%)	
Peritoneal deposits				
Absent	23(46%)	15(65.2%)	8 (34.8%)	0.005
Present	27(54%)	7 (25.9%)	20(74.1%)	
LVI				
Negative	24(48%)	16(66.7%)	8 (33.3%)	0.002
Present	26(52%)	6 (23.1%)	20(76.9%)	
Necrosis				
Absent	19(38%)	9 (47.4%)	10(52.6%)	0.707
Present	31(62%)	13(41.9%)	18(58.1%)	

Chi-square test was used



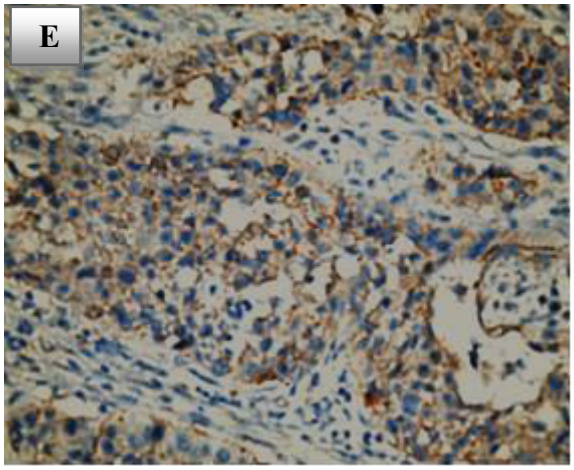


Fig.1. IHC expression of EpCAM in EOC cases: Low EpCAM expression in low-grade ovarian serous carcinoma (a), high EpCAM expression in high-grade ovarian serous carcinoma **(b)**, EpCAM expression in mucinous carcinoma **(c)**, EpCAM expression in ovarian endometrioid carcinoma **(d)**, high EpCAM expression in malignant ovarian Brenner tumor (Transitional cell carcinoma) (IHC; x 200, x 400) **(e).**

Discussion

EOC is a highly aggressive malignant tumor associated with low survival and high mortality rates. This is mainly attributed to associated invasion, metastasis, and chemoresistance. Identification of novel biomarkers related to different invasive properties of ovarian cancer cells is mandatory and, in turn, can improve the clinical outcome in patients with EOC **(Atallah et al., 2021).**

EpCAM has increasingly been recognized both as an oncogene and a CSC marker in various tumors. Earlier research identified EpCAM as a potential prognostic indicator, promoting proliferation, migration, and invasion of cancer cells. Its aberrant expression in tumor cells is associated with different aggressive properties. Furthermore, EpCAM is correlated with resistance to chemotherapy in different tumors (**Mohtar et al., 2020)**. To the best of our knowledge, few previous studies have emphasized the prognostic value of EpCAM expression in EOC.

In this research, EpCAM immunoreactivity was predominantly observed in cell membranes of tumor cells. This was in agreement with the findings of Mohamed and his colleagues, who reported that EpCAM expression was mainly membranous (**Mohamed et al., 2022)**. High EpCAM expression (IRS score ≥6) was detected in 56% of cases, which was consistent with the findings obtained by Tayama et al. and Mohamed et al., who reported high EpCAM expression in 57.7 and 54.7% of the studied cases, respectively **(Tayama et al., 2017; Mohamed et al., 2022).** While higher expression were reported by Spizzo et al. and Zheng et al., who detected high EpCAM expression in 73% and 80% of the studied cases, respectively **(Spizzo et al., 2011; Zheng et al., 2017)**. This variation may be attributed to the difference in sample size.

EpCAM immunoreactivity was different within various histological subtypes of EOC, but this difference was not statistically significant (p=0.058). High EpCAM expression was most frequent in high grade serous carcinoma, while it was least frequent in endometroid carcinoma. This finding was in agreement with the results of Woopen and his colleagues, who found a relationship between

EpCAM expression and histologic subtypes, though with varying frequencies; the highest EpCAM overexpression was noted in endometrioid tumors, and the lowest in mucinous tumors **(Woopen et al., 2014)**. In addition, Spizzo and his colleagues observed that EpCAM expression levels differ depending on the histological subtype of EOC; mucinous carcinoma revealed the lowest rate of EpCAM overexpression compared to other histological subtypes. This difference might be due to the smaller and comparable sample sizes in the current study **(Spizzo et al., 2011)**.

A statistically significant correlation (p=0.033) was found between EpCAM expression and tumor size. High EpCAM expression was more frequent in large-sized tumors (≥10 cm). This finding runs parallel to the findings of **Mohamed et al. (2022).** However, Tayama et al. reported no significant statistical correlation between EpCAM expression and tumor size **(Tayama et al., 2017).**

This study demonstrated a statistically significant correlation between EpCAM expression and tumor laterality (p = 0.013). Bilateral tumors exhibited higher EpCAM expression levels compared to unilateral tumors. In contrast, **Mostafa et al. (2022)** observed no statistically significant correlation between EpCAM expression and tumor laterality, he found that high EpCAM expression was nearly equally distributed between unilateral and bilateral tumor masses. We believe this discrepancy may be attributed to differences in sample size, patient demographics, or methodological variations between the two studies.

The current study revealed a statistically significant correlation between EpCAM expression and tumor grade (p=0.027); high grade tumors expressed higher EpCAM expression than low grade tumors. These findings showed concordance with **Zheng et al. (2017),** who reported similar findings. However, **Woopen et al. (2014)** stated that no relationship existed between EpCAM expression and tumor grade. Furthermore, EpCAM expression analysis demonstrated a significant link between EpCAM expression and FIGO stage (p=0.007); advanced tumor stage was associated with higher of EpCAM expression. This was in line with **Zheng et al. (2017) and Mohamed et al. (2022)**. However, this finding was in disagreement with **Woopen et al. (2014)** and **Tayama et al. (2017)**, who found no statistically significant correlation between EpCAM expression and FIGO stage of EOC.

EpCAM has a critical role in neoplastic cells proliferation, invasion, and migration; emerging evidence suggests that EpCAM enhances both the migration and invasiveness of ovarian cancer cells **(Fagotto and Aslemarz, 2020).** Correlating EpCAM expression with the invasive potential of EOC showed that a consistent rise in EpCAM expression was noted as tumors became increasingly invasive, with a statistically significant positive correlation observed between EpCAM expression and LVI (p= 0.002) and peritoneal deposits (p= 0.005). Mohamed and his colleagues found that cases involving lymphovascular emboli exhibited elevated EpCAM expression. But they reported that metastatic peritoneal deposits showed a direct proportion to EpCAM expression levels, although the correlation was not statistically significant **(Mohamed et al., 2022).**

Conclusion

Increased EpCAM expression in EOC may reflect a more aggressive biological behavior. As result,

targeting this molecule in therapeutic approaches may be a useful strategy to reduce EOC invasion and metastasis.

It is advised to conduct large-scale prospective studies on various histological types of EOC. Monitoring patients is crucial to highlight the relationship between EpCAM expression and patient survival as well as disease outcomes.

Abbreviations list

CSCs: Cancer stem cells
EOC: Epithelial ovarian carcinoma
EpCAM: Epithelial Cell Adhesion Molecule
LNM: Lymph node metastasis
IHC: Immunohistochemistry
LVI: Lymphovascular invasion
PBS: Phosphate-buffered saline
DAB: Diaminobenzidine
IRS: Immunoreactive score.

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Consent to participate: Informed consent was obtained from all individual participant in the study

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