Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2024.38265

Turk J Obstet Gynecol 2024;21:43-50

Evaluation of the diagnostic utility of MCAM-1 (CD146) in a group of common gynecological cancers: A case-control study

Bir grup yaygın jinekolojik kanserde MCAM-1'in (CD146) tanısal faydasının değerlendirilmesi: Bir olgu kontrol çalışması

Amit Kumar[1], Ujjawal Khurana[1], Rashmi Chowdhary[2], Ajay Halder[3], Neelkamal Kapoor[1]

[1]*All India Institute of Medical Sciences, Department of Pathology and Lab Medicine, Bhopal, India*

[2]*All India Institute of Medical Sciences, Department of Biochemistry, Bhopal, India*

[3]*All India Institute of Medical Sciences, Department of Obstetrics and Gynecology, Bhopal, India*

Abstract

Objective: MCAM-1 (CD146) is an endothelial cell adhesion molecule belonging to the immunoglobulin superfamily. Recent studies have identified CD146 expression as a critical marker for tumor progression, migration, and metastasis in various malignancies. This study aimed to evaluate CD146 immunohistochemical expression in various gynecological cancers.

Materials and Methods: This study was conducted in a tertiary medical center in central India. A total of 49 gynecological cancer cases and 16 site-matched controls were included. The cases comprised 27 cervical, 10 endometrial, 10 ovarian, and two miscellaneous cancers. CD146 immunohistochemistry was performed and assessed for immunoreactivity score (IRS), microvascular density (MVD), and microvascular caliber (MVC). An IRS of 5 or more was considered CD146 positive.

Results: The p-values for CD146 positivity for cases vs. control were 0.0531, 0.0580, and 0.007 for cervical, endometrial, and ovarian sites, respectively. The mean MVD was found to be significantly higher in cases compared with benign tissues (p-value <0.00001), and the mean MVC of cases was found to be smaller when compared with the controls (p-value <0.0001).

Conclusion: MVD by CD146 was found to be higher in gynecological malignancies, highlighting its role in cancer neo-angiogenesis and its potential therapeutic role. CD146 epithelial expression was also significantly higher in ovarian cancers. Further studies with a larger sample size are required to confirm that this protein may be a potential theognostic target in gynecological cancers.

Keywords: CD146 antigens, cervical cancer, melanoma cell adhesion molecule, microvascular density, ovarian cancer

Öz

Amaç: MCAM-1 (CD146), immünoglobulin süper ailesine ait bir endotelyal hücre adezyon molekülüdür. Son çalışmalarda, CD146 ekspresyonunun çeşitli malignitelerde tümör progresyonu, migrasyonu ve metastazı açısından kritik bir belirteç olduğu gösterilmiştir. Bu çalışmada çeşitli jinekolojik kanserlerde CD146 immünohistokimyasal ekspresyonunun değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Bu çalışma Hindistan'ın merkezinde üçüncü basamak bir tıp merkezinde gerçekleştirildi. Toplam 49 jinekolojik kanserli hasta ve 16 bölge açısından eşleştirilmiş kontrol dahil edildi. Hastaların 27'sinde rahim ağzı kanseri, 10'unda endometriyal kanser, 10'unda over kanseri ve 2'sinde çeşitli kanserler mevcuttu. CD146 immünohistokimyasal incelemesi yapıldı ve immünoreaktivite skoru (IRS), mikrovasküler yoğunluk (MVD) ve mikrovasküler kalibre (MVC) açısından değerlendirildi. Beş veya daha fazla IRS, CD146 pozitif olarak kabul edildi.

PRECIS: This study is evaluating CD146 expression on a set of gynecological cancers and site-specific controls. CD146 IHC immunoreactivity score, microvessel density and microvessel caliber are evaluated.

Address for Correspondence/Yazışma Adresi: Ujjawal Khurana MD,
All India Institute of Medical Sciences, Department of Pathology and Lab Medicine, Bhopal, India
Phone: +917773010092 **E-mail:** ujjawal.patho@aiimsbhopal.edu.in **ORCID ID:** orcid.org/0000-0003-3913-7111

Received/Geliş Tarihi: 09.12.2023 **Accepted/Kabul Tarihi:** 04.02.2024

Bulgular: Kontroller ile kıyaslandığında hastalarda CD146 pozitifliğinin p-değerleri servikal, endometrial ve over bölgeleri için sırasıyla 0,0531, 0,0580 ve 0,007 idi. Ortalama MVD hasta dokularda benign dokulara göre anlamlı derecede yüksek (p-değeri <0,00001) bulunurken, hastaların ortalama MVC'si ise kontrollere göre daha küçük bulundu (p-değeri <0,0001).

Sonuç: CD146 ile MVD'nin jinekolojik malignitelerde daha yüksek olduğu bulundu, bu da onun kanser neo-anjiyogenezindeki rolünü ve potansiyel terapötik rolünü vurgulamaktadır. Yumurtalık kanserlerinde CD146 epitel ekspresyonu da anlamlı derecede yüksekti. Bu proteinin jinekolojik kanserlerde potansiyel bir tanısal hedef olabileceğini doğrulamak için daha büyük örneklem büyüklüğüne sahip çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: CD146 antijenleri, rahim ağzı kanseri, melanom hücre yapışma molekülü, mikrovasküler yoğunluk, yumurtalık kanseri

Introduction

Gynecological cancers (cervix, endometrium, and ovaries) are a significant global burden, with 13.9 lakh new cases and 6.7 lakh deaths reported in 2020[1]. Diagnosis of gynecological cancers involves clinical examination findings, radiological imaging, and histopathological examination[2,3]. The prognosis depends on the site of gynecological cancer, its staging, histological subtype, grade, etc. Biomarkers are measurable biological substances for screening and monitoring of occult gynecological tumors, such as CA-125, CA 19.9, CEA, and HE-4, which have clinical utility in ovarian and endometrial cancers[4].

Melanoma cell adhesion molecule, also known as cluster of differentiation (differentiation), is an endothelial cell adhesion molecule belonging to an immunoglobulin superfamily[5,6]. Lehmann et al.[4] first studied it in malignant melanomas in 1987. It is a membrane glycoprotein expressed by endothelial cells, T-cells, Schwann cells, etc. and plays a pivotal role in vessel formation, immune response, and nerve regeneration[7,8]. Recent studies have found CD146 expression to be a key marker for tumor angiogenesis, progression, migration, epithelial-mesenchymal transition, and metastasis in various malignancies such as melanoma, breast, malignant pleural mesothelioma, prostate, lung, gastric, and gallbladder cancers[5-9]. Increased CD146 expression is strongly correlated with poor prognosis[6]. CD146 is considered a superior marker for disease progression compared with sentinel lymph node analysis. The expression of this protein has been assessed by various methods such as immunohistochemistry (IHC), immunofluorescence, western blotting, and quantitative polymerase chain reaction[5,10,11]. CD146 immunohistochemical expression has been observed in 43% of cervical cancers, 67% of endometrial cancers, and approximately 50% of epithelial ovarian cancers[5,11,12].

This research aims to assess CD146 expression in gynecological cancers compared with benign controls and determine its association with tumor subtypes, biological features, and clinical characteristics. Microvascular density (MVD) and mean vascular caliber (MVC) on CD146 IHC are also explored.

Materials and Methods

This case-control study was conducted in the Department of Pathology and Laboratory Medicine after obtaining ethical clearance from the Institutional Ethics Committee (LOP No IHEC-PGR/2022/STS-ICMR/10, date: 20.06.2022). Waiver of consent was granted because patient identifiers were not used and confidentiality was assured. The study was conducted in accordance with the ethical standards described in the Declaration of Helsinki.

Records, forms, slides, and blocks of diagnosed gynecological cancer cases were retrieved from the archives. The inclusion criteria were cases diagnosed as carcinoma cervix, carcinoma endometrium, and epithelial ovarian malignancies. Suboptimal quality blocks were excluded; cases that had received prior chemotherapy and radiotherapy were also excluded. The sample population included 49 cases and 16 site-matched benign controls: Twenty-seven cases of cervical cancer, 10 cases each of endometrial and ovarian cancers, and 2 cases of miscellaneous cancers. Five cases of chronic cervicitis, 5 benign ovarian tissues, 5 benign endometrial tissues, and one case of tubectomy specimen formed respective benign tissue controls.

CD146 expression, MVD, and MVC were evaluated in these malignancies and compared with site-matched benign controls.

IHC Staining: Representative blocks were selected and stained. Staining was compared with known positive and negative controls. Human placental tissue and melanoma cases were used as positive controls, and omission of primary antibody was used as negative controls. CD146 IHC was performed on tissue blocks with antigen retrieval using Tri sodium citrate buffer. Primary antibody CD146 [Biogenex, Anti Human CD146 (clone EP54)] was applied, followed by washing and incubation with Polyexcel target binder and Polyexcel PolyHRP (horse radish Peroxidase). DAB solution (1 mL of stunt DAB buffer with one drop of Stunn DAB chromogen) was added, counterstaining with hematoxylin, dehydration, and mounting with DPX.

CD146 Expression Criteria: Each biopsy was evaluated for immunoreactivity score (IRS) by assessing staining intensity (0=no staining, 1=faint staining, 2=moderate staining, 3=intense staining) and estimated percentage of all positive cells (0=non reacting cells, 1=1-10% reacting cells, 2=11-25% reacting cells, 3=26-50% reacting cells, 4≥50% reacting cells)[11].

An IRS of 5 or more was considered positive. The cellular localization of CD146 on the cell membrane, nuclear membrane, and nucleoli was also recorded.

MVD and MVC: MVD was analyzed using a CILIKA microscope with morphometric analysis. CD146-immunostained sections were captured, and vessel counting was performed using appropriate reference criteria[12-14]. Three hotspots with the highest microvessel count were determined at low power. These areas were then observed at high magnification (x400) and the average value of 3 hotspot areas was taken as the representative

value of tumor MVD. The high magnification (x400) has a field diameter of 450 µm (0.45 mm) and a field area of approximately 160.000 µm^2 (0.16 mm^2). The MVC was also measured at high magnification.

CD146 in Relation to Diagnosis and Prognosis: We also looked for its relationship with tumor depth in cervical and endometrial variants, along with any correlation with International Federation of Gynaecology and Obstetrics (FIGO) or TNM staging.

Statistical Analysis

We analyzed data using SPSS software. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), diagnostic accuracy (DA), and likelihood ratios were calculated using standard formulas. The significant differences between the groups and the association between CD146 expression and different clinicopathological parameters were evaluated using the Fisher's Exact test. The Kruskal-Wallis test was used to compare the different grades of cancer with their benign counterparts, and an unpaired t-test was used to evaluate the association of MVC. A p-value of <0.05 was considered statistically significant.

Observations and Results

In our study, we analyzed 49 cases and 16 controls, evaluating CD146 expression, MVD, and MVC in cervical cancer (27), endometrial cancer (10), ovarian cancer (10) and one case each of serous tubal intraepithelial carcinoma (STIC) (1) and vulval cancer (1).

Cervical Cancer

All patients with cervical cancer included in our study underwent cervical biopsy, and the biopsy tissue was used for IHC staining. The age of the patients in the cervical cancer group ranged from 37 to 82 years, with an average of 54.51 years. The maximum number of patients was observed in the fourth and fifth decade of life.

Of the 27 cases, 25 were squamous cell carcinoma (SCC) and 2 were adenocarcinoma. In SCC, 17 (68%) cases were moderately differentiated (MDSCC), 5 (20%) cases were poorly differentiated SCC (PDSCC), and 3 (12%) cases were well differentiated SCC (WDSCC).

Nineteen (70%) cases showed CD146 expression, and eight (30%) cases were negative (Figure 1a-1d). In the cervical benign cases, 1 (20%) was positive and the remaining 4 (80%) were negative. The p-value was 0.0531 and was non-significant (Fisher's Exact test) (Table 1). If we consider CD146 positivity of WDSCC in comparison with the rest of SCC, it was 33% in the former and 68% in the latter.

Among all SCCs, the MVD ranged from 4 to 36 in different hotspots with a mean of 14.316±6.64 per 0.16 mm^2, whereas in benign tissues, MVD ranged from 3 to 12 with a mean of 5.53±2.02 per 0.16 mm^2 as explained in Table 1. The p-value was <0.00001 and was significant (Kruskal-Wallis test).

In WDSCC, the MVD ranged from 7 to 16 per 0.16 mm^2 (mean 10.77±0.69). In MDSCC, MVD ranged from 5 to 36 per 0.16 mm^2 (mean 14.505±8.8). In PDSCC, the MVD ranged from 4 to 25 per 0.16 mm^2 (mean 10.13±5.24). Median MVD values were 33.31, 68.75, 87.5, 81.25 per mm^2 for benign cervical tissue, WDSCC, MDSCC, and PDSCC, respectively (Figure 2). MVD was highest in MDSCC, followed by PDSCC and WDSCC, possibly due to increased necrosis and hemorrhage in PDSCC.

The mean MVC in cervical cancers was 9.824±4.873 µm, while in benign tissue it was 31.8±15.380 µm (Table 1). The difference was statistically significant (p<0.0001) based on an unpaired t-test.

Most cervical cancer types were SCC, followed by adenocarcinoma. Among the 11 cases with available FIGO staging, 9 (77%) in the early stage (I-II) and 2 (100%) in the advanced stage (III-IV) showed positive CD146 expression.

Endometrial Carcinoma

Ten random cases of endometrial carcinoma, endometrial tissue used for IHC was of the 6 patients who had undergone total abdominal hysterectomy and bilateral salpingo-oophorectomy, and 4 were endometrial curettage.

The age of patients in the endometrial cancers group ranged from 47 to 76 years, with a mean age of 58 years. Maximum number of patients presented in the 5th decade. In carcinoma of the endometrium, 7 (70%) cases were low-grade endometrial carcinoma (LGEC), 2 (20%) cases were high-grade endometrial carcinoma (HGEC), and 1 (10%) was mucinous endometrial adenocarcinoma.

Five (50%) cases showed CD146 expression, whereas the remaining 50% were negative (Figure 1e-1h). Among the benign group, 20% were positive and 80% were negative. The Fisher's Exact test statistic value was 0.5804, indicating a non-significant result (Table 1). Among the 5 positive cases, 2 were HGEC and 3 were LGEC.

In all cases of endometrial carcinomas, MVD ranged from 9 to 32 (mean 17.83±6.07) per 0.16 mm^2. In benign tissues, MVD ranged from 8 to 22 (mean 12.25±3.88) per 0.16 mm^2 (Table 1, Figure 2). The p-value obtained from the Kruskal-Wallis test was 0.001, indicating a significant difference (p<0.05) between the two groups.

In HGEC, the MVD ranged from 14 to 28 (mean 20.83±4.002) per 0.16 mm^2. In LGEC, MVD ranged from 11 to 38 (mean 17.75±6.64) per 0.16 mm^2. In mucinous adenocarcinoma, MVD ranged from 10 to 16 (mean 13.33±3.05) per 0.16 mm^2. In the normal endometrium, MVD ranged from 8 to 22 (mean 12.24±3.89) per 0.16 mm^2. The median MVD values per mm^2 were 70.62, 87.5, and 130.185 for benign, LGEC, and HGEC, respectively (Figure 2). Median MVD showed an increase with higher histological grade, with the highest in HGEC, compared with benign tissue.

The mean MVC in endometrial cancers was 6.52±2.311 µm, while in benign tissue it was 22.23±10.150 µmm. The difference was extremely statistically significant (p<0.0001) based on an unpaired t-test.

The majority of endometrial carcinomas in the study were endometrioid adenocarcinomas. Among the six cases with available FIGO staging, two (50%) were in stage I (early stage; FIGO I-II) and four (75%) were in advanced stage (III-IV) with positive CD146 expression. CD146 expression increased with the stage of endometrial carcinoma.

Ovarian Carcinoma

Ten cases meeting the inclusion criteria and suitable blocks were selected. Samples included were from the following surgical procedures: total abdominal hysterectomy with bilateral salpingo-oophorectomy (7), right oophorectomy (1), ovarian cystectomy (1), and excised ovarian mass (1).

Eight were serous and two mucinous. Two cases were atypical proliferative serous tumors (APST), one was a case of low-grade serous carcinoma (LGSC), and five were high-grade serous carcinoma (HGSC). In the mucinous group, both were mucinous ovarian carcinoma (MOC). All epithelial ovarian tumors are referred to as ovarian cancers in this study. Patients' age ranged from 19 to 65 years (mean: 39.4 years). There were 1, 3, 1, 2, 2 & 1 cases within the age group of 11 to 20 years, 21 to 30 years, 31 to 40 years, 41 to 50 years, 51-60 years, and more than 60 years.

In the evaluated epithelial ovarian tumors, 80% of cases were CD146 positive (Figure 1j-1l), whereas 20% were negative. Among the controls, all were negative. Out of the ten cases, five were HGSC with 80% positive (4) and 20% negative (1). Both cases of MOC were positive (100%), one LGSC was positive (100%), and two APST cases had one positive (50%) and one negative (50%). DA was 86.67%, sensitivity was 80%, specificity was 100%, PPV was 100%, NPV was 71.43%, and likelihood ratio was 0.20 (Table 1). The Fisher's Exact test statistic value is 0.007. The result is significant at $p < 0.05$.

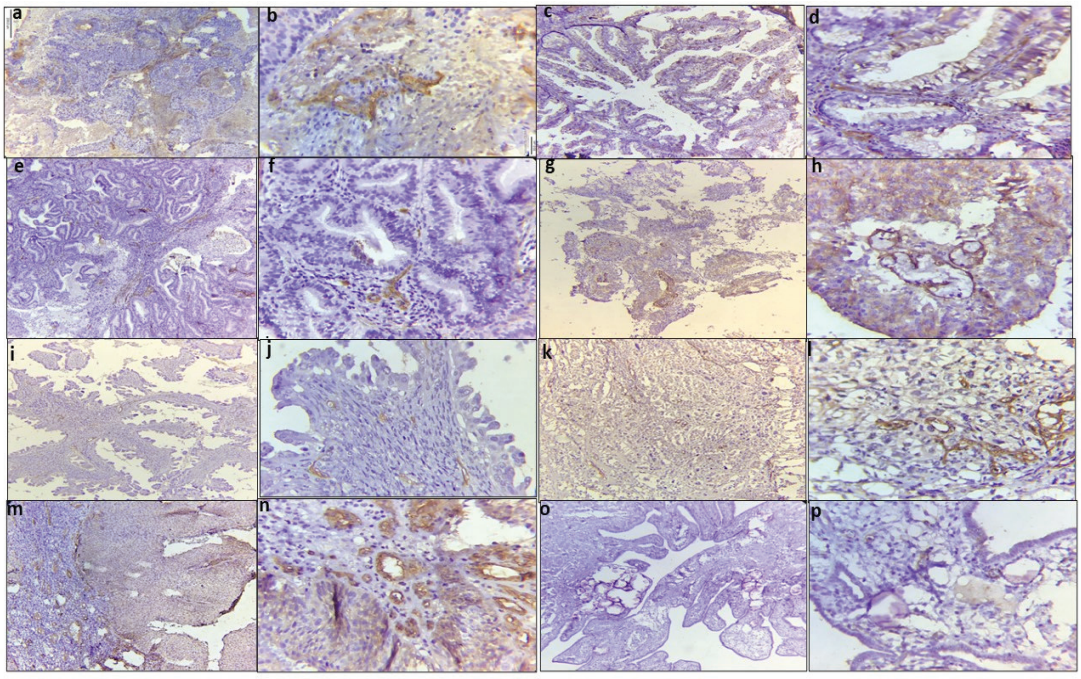

Figure 1. Photomicrographs 'a' (at low power) & 'b' (at high power with a mean MVD of 62.5 per mm^2 per mm^2) show a case of WDSCC, with an IRS of 5 i.e., Positive for CD146 expression; whereas 'c' (at low power) & 'd' (at high power with a mean MVD of 133.312 per mm^2) show a case of endocervical adenocarcinoma, with an IRS of 6 i.e., Positive for CD146 expression

Photomicrographs 'e' (at low power) & 'f' (at high power with mean MVD of 95.81 per mm^2) showing a case of LGEC with an IRS of 3 i.e., negative for MCAM expression whereas 'g' (at low power) & 'h' (at high power with a mean MVD of 147.87 per mm^2) show a case of HGEC with an IRS of 6 i.e., positive for CD146 expression.

Photomicrographs 'i' (at low power) & 'j' (at high power with a mean MVD of 45.81 per mm^2) showing a case of APST with an IRS of 4 i.e., negative for CD146 expression; whereas 'k' (at low power) & 'l' (at high power with a mean MVD of 197.87 per mm^2) showing a case of MOC with an IRS of 6 i.e., positive for CD146 expression.

Photomicrographs 'm' (at low power) & 'n' (at high power with a mean MVD of 231.25 per mm^2) show a case of squamous carcinoma of the vulva with an IRS of 6 i.e., is positive for CD146 expression.

Photomicrograph 'o' (at low power) & 'p'(at high power with a mean MVD of 133.312 per mm^2) showing a case of STIC with an IRS of 3 i.e. Negative for CD146 expression

MOC: Mucinous ovarian carcinoma, MVD: Microvascular density, IRS: Immunoreactivity score, HGEC: High grade endometrial carcinoma, APST: Atypical proliferative serous tumour, STIC: Serous tubal intraepithelial carcinoma

Table 1. Distribution of CD146 expression, MVD, MVC in gynecological cancers and benign controls of the indexed study

Site	Cervical		Endometrial		Ovarian	
Variables	Cancers (n=27)	Benign (n=5)	Cancers (n=10)	Benign (n=5)	Cancers (n=10)	Benign (n=5)
CD 146^{+}	19	1	5	1	8	0
CD 146^{-}	8	4	5	4	2	5
p-value	0.0531		0.5804		0.007	
DA	71.8		50		86.67	
Sensitivity	70		50		80	
Specificity	80		80		100	
PPV	95		83.3		100	
NPV	33.33		44.4		71.43	
LR+	3.52		2.5		-	
LR-	0.37		0.62		0.20	
Mean MVD	14.316±6.64	5.53±2.02	17.83±6.07	12.25±3.88	18.53±8.65	6.73±1.090
p-value	<0.00001		0.0010		0.0001	
Mean MVC (µm)	9.824±4.873	31.8±15.380	6.52±2.311	22.2±10.150	7.1±2.682	13.06±2.300
p-value	0.0001		0.0001		0.0001	

DA: Diagnostic accuracy, PPV: Positive predictive value, NPV: Negative predictive value, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, MVD: Microvascular density, MVC: Microvascular caliber

In ovarian cancers, MVD ranged (Table 1, Figure 2) from 6 to 42 (mean: 18.53±8.653 per 0.16 mm^2), whereas in benign tissue, it ranged from 4 to 12 (mean: 6.73±1.090 per 0.16 mm^2). The p-value (0.0001) indicates significance (p<0.05) based on the Kruskal-Wallis test.

In HGSC, the MVD ranged from 8 to 27 (mean: 18.13±4.793 per 0.16 mm^2). In APST, MVD ranged from 6 to 13 (mean: 9.16±2.595 per 0.16 mm^2). In MOC, MVD ranged from 13 to 42 (mean: 25.66±8.485 per 0.16 mm^2). In LGSC, the MVD ranged from 26 to 35 (mean: 29.66 per 0.16 mm^2). Median MVD per mm^2 were 39.56, 55.78, 185.37, 113.32, and 160.37 for benign, APST, LGSC, HGSC, and MOC, respectively (Figure 2). Among ovarian carcinomas, MVD was lowest in APST and highest in LGSC.

In ovarian cancers, the mean MVC was found to be 7.1±2.682 µm whereas in benign tissue, it was 13.06±2.300 µm as given in Table 1. The two-tailed p-value is less than 0.0001, making the difference extremely statistically significant (calculated by using an unpaired t-test).

Most ovarian cancers were epithelial. Among the ten cases, four early-stage (I-II) and six advanced-stage (III-IV) cases showed positive CD146 expression. CD146 expression increased with higher stages of ovarian carcinoma. MVD was higher in ovarian carcinomas than in benign controls and borderline cases.

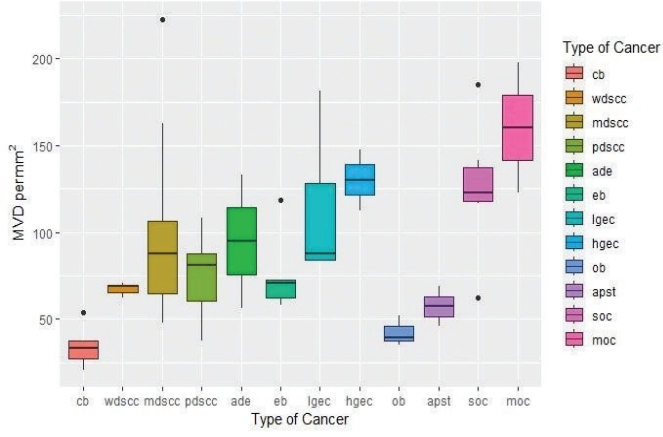

Figure 2. Box and whisker plot showing mean microvascular density in cases and controls of the cervical cancer, endometrial cancer, ovarian cancer

cb: Cervical benign tissue, wdscc: Well differentiated squamous cell carcinoma, mdscc: Moderately differentiated squamous cell carcinoma, pdscc: Poorly differentiated squamous cell carcinoma, ade: Adenocarcinoma cervix, eb: Endometrial benign tissue, lgec: Low grade endometrial carcinoma, hgec: High grade endometrial carcinoma, ob: Ovarian benign tissue, apst: Atypical proliferative serous tumour, soc: Serous ovarian carcinoma, moc: Mucinous ovarian carcinoma

Table 2. A comparison of MVD of common gynecological cancers as depicted in literature along with this indexed study

Author	Study location	Tissue	IHC Marker	Sample Size	Mean MVD	Magnification (Field area)
Index article	India	Cervical cancer	CD146	27	14.316± 6.64	400X (0.16 mm^2)
Liu et al. (2013)[16]	China	Cervical cancer	CD34	56	WDSCC: 30.83±2.98 MDSCC: 43.86±3.92 PDSCC: 54.19±5.36	200X (NA)
Triratanachat et al.[18] (2006)	Thailand	Cervical cancer (SCC)	CD31	60	54.87±25.5	400X (0.085 mm^2)
Index study	India	Endometrial cancer	CD146	10	17.83±6.07	400X (0.16 mm^2)
Kluz et al.[20] (2020)	Poland	Endometrial cancer	CD34	117	19 (Median)	200X
Haldorsen et al.[21] (2013)	Norway	Endometrial cancer	Factor VIII	54	49	250X (0.424 mm^2)
Index study	India	Ovarian cancer	CD146	10	18.53 ± 8.653 per 0.16 mm^2	400X (0.16 mm^2)
Onisim et al.[12] (2019)	Romania	Epithelial ovarian cancer	CD146 & CD34	101	44.73±21.44 by CD146 46.72±22.29 by CD34	400X
Sopo et al.[13] (2020)	Finland	Epithelial ovarian cancers	CD34, CD105 and D2-40	86	340.69±18.5 per mm^2 by CD34 93.94±6.12 Per mm^2 by CD105	200X

WDSCC: Well differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma; PDSCC: Poorly differentiated squamous cell carcinoma, MVD: Microvascular density

Miscellaneous

Two cases were included: one vulval carcinoma and one STIC. The vulval carcinoma was CD146 positive (IRS 6) with a mean MVD of 37 vessels per 0.16 mm^2 (Figure 1m-1n). The STIC case was CD146 negative (IRS 4) with a mean MVD of 21.33 vessels per 0.16 mm^2 (Figure 1o-1p), whereas the normal fallopian tube had an IRS of 3 and a mean MVD of 11.66 vessels per 0.16 mm^2.

Discussion

In our study, we observed that epithelial expression of the CD146 marker is a reliable tool for differentiating ovarian cancers from benign controls, exhibiting a sensitivity, specificity, and DA of 100% each. However, in the case of endometrial and cervical cancers, CD146 does not serve as a suitable marker for diagnostic utility. However, MVD on CD146 was significantly different in all forms of gynecological cancers compared with benign controls.

Our study found CD146 expression in 70% of cervical cancers, which is slightly higher than a report of 49% in a study by Zhang et al.[5] In addition, a study on assessment of multiple markers in trophoblastic tumors and cervical cancers reported CD146 expression in 20% cervical carcinoma cases[15]. CD146 expression was higher in cervical cancers but was not statistically significant. Among cervical cancer subtypes, MDSCC showed the highest expression, followed by PDSCC and WDSCC. Liu et al.[16] performed MVD on CD34 and VEGF and observed that expression in SCC progressively increased with the grade of cancer (highest in PDSCC & lowest in WDSCC) and it was found to be statistically significant. Whereas we found that the mean of MVD was highest for MDSCC followed by WDSCC and PDSCC; this difference may be due to the abundance of hemorrhage and necrosis in PDSCC and because we have taken cervical biopsy specimens. Higher vascular density was associated with poorer prognosis in one study, whereas there was no statistical difference in outcomes based on MVD[17,18]. Hu et al.[19] conducted a meta-analysis and suggested that a high count of MVD is associated with poor survival among cervical cancers. MVC was significantly less in cervical carcinoma cases than in benign biopsies.

Our study found CD146 expression in 50% of endometrial cancers, and it has been previously reported as 67%[5]. CD146 expression in endometrial carcinomas was higher in endometrial adenocarcinoma and mucinous compared with that in benign tissues. CD146 was highly expressed in advanced stages (FIGO III-IV), in line with Zhang et al.[5] Mean MVD was higher for endometrial carcinoma compared with that in benign tissue, and within the carcinomas, higher mean MVD was found in HGEC compared with that in LGEC. Similar trends were found in the study by Kluz et al.[20], who also found no relation between FIGO stage of EC and MVD. Haldorsen et al.[21] found that high MVD was significantly associated with reduced recurrence-

free. We found a decreasing trend of MVC in malignant when compared to borderline and benign, and it was significant with a p-value <0.05 but as of our knowledge not much studies have been conducted on MVC in endometrial cancers.

CD146 was significantly more expressed in EOC and MOC than in benign tissue, with a positivity of 80%. Advanced stages of ovarian carcinomas showed increased CD146 expression, but it was not statically significant, in line with Onisim et al.[12] They reported nearly 50% positivity and observed that CD146 can be used as an independent prognostic marker indicating a disease requiring intensive management and increased chances of chemotherapy resistance. They also mentioned that CD146 may be regarded as a novel biomarker of tumor neovasculature and found it to be as reliable as CD34 for MVD estimation. Another study conducted on 133 cases of epithelial ovarian carcinoma found CD146 expression in 65 cases (48.8%). They found that the expression of this molecule was significantly associated with advanced stage (p=0.0001), serous and undifferentiated phenotype (p=0.0004), accumulated p53 (p=0.0002) and residual tumour(p=0.0015)[11]. Our study found a significantly higher CD146 positivity of 80%. High CD146 positivity may suggest a more aggressive form of the disease, which could influence treatment strategies and the need for more intensive management.

Sopo et al.[13] assessed MVD on endoglin and CD34 by IHC and found that MVD was significantly higher in ovarian malignancy and borderline tumors as compared to benign. They also found that high MVD assessed by CD34 had a longer progression-free interval, and this trend was opposite with CD105[13]. In a meta-analysis of 22 studies done on MVD in ovarian cancer, they found that the OS and PFS with high MVD were significantly poorer than with low MVD in ovarian cancer patients[22]. However, high MVD detected by CD34 seems to be more associated with survival in patients without pre-chemotherapy. MVC assessed by CD34 was significantly different between benign and borderline/malignant tumors. We found a decreasing trend of MVC in malignant tumors when compared with borderline and benign tumors, and it was significant with a p-value <0.05. As tubal, vulval, and vaginal are rare gynecological cancers, accounting for less than 5% of all gynecological cancers[1,2], only one case for tubal or vulval cancer could be retrieved. CD146 positivity with high MVD was observed in squamous vulval carcinoma. STIC showed CD146 negativity and high MVD.

Angiogenesis plays a vital role in the growth, metastasis, and progression of invasive carcinomas. Tumors >1 mm require angiogenesis for further growth, leading to metastasis[19,23]. High MVD promotes local tumor propagation and hematogenous spread, worsening prognosis[17]. CD146 demonstrates potential utility as a preoperative marker in biopsy samples[19]. A comparison of MVD of common gynecological cancers as depicted in literature along with this indexed study is depicted in Table 2[12,13,16,18,20,21]. High MVD detected with CD146 can inform surgical decisions, facilitating pelvic node resection in addition to tumor removal. This approach may enhance the precision and effectiveness of surgical interventions in gynecological malignancies. MVD was significantly higher in malignant tissues of cervical, endometrial, and ovarian origin, supporting the role of neo-angiogenesis in malignancy and metastatic potential and eventual poorer survival[24-26]. Vessel size in gynecological cancers of the study was significantly smaller compared with benign controls, regardless of cancer type or grade, aligning with previous studies on complex and intricate vasculature in malignancies[14,16].

CD146 positivity was extensively evidenced in the tumor cell membrane in some cases and in the endothelial cell cytoplasm of microvessels in the tumor stroma. This expression in the tumor cells and stroma opens multiple perspectives regarding the possible implications of this cell adhesion molecule in tumor development, growth, epithelial-mesenchymal transition, and progression. CD146 has also been associated with poor survival in human solid tumors, establishing its role as a prognostic predictive biomarker, but its role as a potential therapeutic target in human solid tumors needs to be further studied[25,26].

Study Limitations

Although the spectrum of gynecological cancers taken was more, the indexed study had a limitation of a small sample size for endometrial and ovarian cancers. CD146 epithelial expression did not differ significantly between benign and malignant tissues among cervical and endometrial carcinomas, whereas it was significant in ovarian carcinomas and borderline tumors as compared to benign tissues.

Future scopes include enhancing CD146-specific detection methods for cervical and endometrial cancers, exploring anti-CD146 therapy for ovarian cancer treatment, and investigating anti-CD146 immunotherapy in combination with traditional treatments as a promising anticancer approach in gynecological cancers[23,27]. This also implies that a combined treatment strategy of anti-CD146 immunotherapy with other traditional chemo- or radiotherapy treatments may be a promising anticancer technique.

Conclusion

To summarize, CD146 epithelial expression was significant in ovarian cancers, and malignant tissues exhibited higher MVD when assessed with CD146 staining, suggesting increased neoangiogenesis. Furthermore, we observed that the vessel caliber is narrower in gynecological malignancies. Overall, CD146 is a promising and reliable marker for assessing MVD in gynecological malignancies. The presence of increased MVD has the potential to enhance clinical management decisions when diagnosing gynecological malignancies. This information could aid in refining treatment strategies and improving patient outcomes.

Ethics

Ethics Committee Approval: This case-control study was conducted in the Department of Pathology and Laboratory Medicine after obtaining ethical clearance from the Institutional Ethics Committee (LOP No IHEC-PGR/2022/STS-ICMR/10, date: 20.06.2022 - All India Institute of Medical Sciences, BHOPAL).

Informed Consent: Waiver of consent was granted because patient identifiers were not used and confidentiality was assured.

Authorship Contributions

Surgical and Medical Practices: U.K., A.H., Concept: A.K., U.K., R.C., N.K., Design: A.K., U.K., R.C., Data Collection or Processing: A.K., U.K., A.H., Analysis or Interpretation: A.K., U.K., N.K., Literature Search: A.K., U.K., R.C., A.H., N.K., Writing: A.K., U.K., R.C., A.H., N.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. World Health Organization. Factsheets. 2022. Available from: https://gco.iarc.fr/today/fact-sheets-cancers
2. Kumar S, Padubidri, VG, Daftari SN. Howkins and Bourne Shaw's Textbook of Gynaecology, 17th ed. New Delhi: Elsevier; 2018.
3. Tanyi JL, Scholler N. Oncology biomarkers for gynecologic malignancies. Front Biosci 2012;4:1097-110.
4. Lehmann JM, Holzmann B, Breitbart EW, Schmiegelow P, Riethmüller G, Johnson JP. Discrimination between benign and malignant cells of melanocytic lineage by two novel antigens, a glycoprotein with a molecular weight of 113,000 and a protein with a molecular weight of 76,000. Cancer Res 1987;47:841-5.
5. Zhang H, Zhang J, Wang Z, Lu D, Feng J, Yang D, et al. CD146 is a potential marker for the diagnosis of malignancy in cervical and endometrial cancer. Oncol Lett 2013;5:1189-94.
6. Stalin J, Nollet M, Dignat-George F, Bardin N, Blot-Chabaud M. Therapeutic and Diagnostic Antibodies to CD146: Thirty Years of Research on Its Potential for Detection and Treatment of Tumors. Antibodies (Basel) 2017;6:17.
7. Pickl WF, Majdic O, Fischer GF, Petzelbauer P, Faé I, Waclavicek M, et al. MUC18/MCAM (CD146), an activation antigen of human T lymphocytes. J Immunol 1997;1:158.
8. Ouhtit A, Gaur RL, Abd Elmageed ZY, Fernando A, Thouta R, Trappey AK, et al. Towards understanding the mode of action of the multifaceted cell adhesion receptor CD146. Biochim Biophys Acta 2009;1795:130-6.
9. Sato A, Torii I, Okamura Y, Yamamoto T, Nishigami T, Kataoka TR, et al. Immunocytochemistry of CD146 is useful to discriminate between malignant pleural mesothelioma and reactive mesothelium. Mod Pathol 2010;23:1458-66.
10. Zhou P, Xiong T, Chen J, Li F, Qi T, Yuan J. Clinical significance of melanoma cell adhesion molecule CD146 and VEGFA expression in epithelial ovarian cancer. Oncol Lett 2019;17:2418-24.
11. Aldovini D, Demichelis F, Doglioni C, Di Vizio D, Galligioni E, Brugnara S, et al. M-CAM expression as marker of poor prognosis in epithelial ovarian cancer. Int J Cancer 2006;119:1920-6.
12. Onisim A, Vlad C, Simon I, Dina C, Achimas Cadariu P. The role of CD146 in serous ovarian carcinoma. J BUON 2019;24:1009-19.
13. Sopo M, Anttila M, Muukkonen OT, YlÄ-Herttuala S, Kosma VM, Keski-Nisula L, et al. Microvessels in Epithelial Ovarian Tumors: High Microvessel Density Is a Significant Feature of Malignant Ovarian Tumors. Anticancer Res 2020;40:6923-31.
14. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis correlation in invasive breast carcinoma. N Engl J Med 1991;3:324.
15. Kalhor N, Ramirez PT, Deavers MT, Malpica A, Silva EG. Immunohistochemical studies of trophoblastic tumors. Am J Surg Pathol 2009;33:633-8.
16. Liu Y, Ye Z, Sun H, Bai R. Grading of uterine cervical cancer by using the ADC difference value and its correlation with microvascular density and vascular endothelial growth factor. Eur Radiol 2013;23:757-65.
17. Höckel S, Schlenger K, Vaupel P, Höckel M. Association between host tissue vascularity and the prognostically relevant tumor vascularity in human cervical cancer. Int J Oncol 2001;19:827-32.
18. Triratanachat S, Niruthisard S, Trivijitsilp P, Tresukosol D, Jarurak N. Angiogenesis in cervical intraepithelial neoplasia and early-staged uterine cervical squamous cell carcinoma: clinical significance. Int J Gynecol Cancer 2006;16:575-80.
19. Hu X, Liu H, Ye M, Zhu X. Prognostic value of microvessel density in cervical cancer. Cancer Cell Int 2018;18:152.
20. Kluz T, Łoziński T, Czekierdowska S, Stachowicz N, Gurynowicz G, Chróściel M, et al. Tumor budding index and microvessel density assessment in patients with endometrial cancer: A pilot study. Oncol Lett 2020;20:2701-10.
21. Haldorsen IS, Stefansson I, Grüner R, Husby JA, Magnussen IJ, Werner HM, et al. Increased microvascular proliferation is negatively correlated to tumor blood flow and is associated with unfavourable outcome in endometrial carcinomas. Br J Cancer 2014;110:107-14.
22. He L, Wang Q, Zhao X. Microvessel density as a prognostic factor in ovarian cancer: a systematic review and meta-analysis. Asian Pac J Cancer Prev 2015;16:869-74.
23. Cheng H. Inhibiting CD146 by its Monoclonal Antibody AA98 Improves Radiosensitivity of Cervical Cancer Cells. Med Sci Monit 2016;22:3328-33.
24. Rubatt JM, Darcy KM, Hutson A, Bean SM, Havrilesky LJ, Grace LA, et al. Independent prognostic relevance of microvessel density in advanced epithelial ovarian cancer and associations between CD31, CD105, p53 status, and angiogenic marker expression: A Gynecologic Oncology Group study. Gynecol Oncol 2009;112:469-74.
25. Zeng P, Li H, Lu PH, Zhou LN, Tang M, Liu CY, et al. Prognostic value of CD146 in solid tumor: A Systematic Review and Meta-analysis. Sci Rep 2017;7:4223.
26. Zhou Y, Huang H, Yuan LJ, Xiong Y, Huang X, Lin JX, et al. CD146 as an adverse prognostic factor in uterine sarcoma. Eur J Med Res 2015;20:67.
27. Ma X, Wang J, Liu J, Mo Q, Yan X, Ma D, et al. Targeting CD146 in combination with vorinostat for the treatment of ovarian cancer cells. Oncology Letters 2017;13:1681-7.