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Original Research Article

IMMUNOHISTOCHEMICAL EVALUATION OF TUMOUR ANGIOGENESIS IN OVARIAN SURFACE EPITHELIAL TUMOURS: DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS

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ABSTRACT

Background: Ovarian surface epithelial tumours (OSETs) constitute the majority of ovarian neoplasms, exhibiting a wide biological spectrum from benign to malignant forms. Angiogenesis and vascular maturity play pivotal roles in their growth and metastatic potential. This study evaluated angiogenic activity using microvessel density (MVD) and vessel maturity using smooth muscle actin (SMA) expression across different histological subtypes of OSETs. **Aim and Objectives:** To determine the incidence of OSETs at a tertiary care hospital; to compare angiogenesis between benign, borderline, and malignant epithelial tumours using immunohistochemistry (CD34 and SMA); and to assess the characteristics of tumour vasculature. **Materials and Methods:** An observational cross-sectional study was conducted on 50 histopathologically confirmed OSETs received at the Department of Pathology. Formalin-fixed, paraffin-embedded sections were stained with haematoxylin and eosin for histopathology and subjected to immunohistochemistry using anti-CD34 and anti-SMA antibodies. MVD was assessed by counting endothelial "hot spots" on CD34-stained sections, and SMA expression index was calculated as the ratio of mature vessels showing smooth muscle coverage. Statistical analyses were performed using Student's *t*-test and Pearson correlation. **Result:** The incidence of OSETs was 61.36%. Serous tumours were the most common (56%), followed by mucinous (40%) and Brenner (4%). Mean MVD increased significantly from benign (4.48 ± 0.99) to malignant tumours (16.53 ± 2.36) ($t(44) = 18.54$, $p < 0.001$). Conversely, the mean SMA expression index decreased from benign (0.48 ± 0.08) to malignant lesions (0.17 ± 0.03) ($t(44) = 19.37$, $p < 0.001$). A strong negative correlation was observed between MVD and SMA index ($r = –0.78$, $p < 0.001$). **Conclusion:** Malignant OSETs demonstrate significantly higher angiogenesis and reduced vessel maturity compared with benign tumours. Combined assessment of MVD and SMA expression serves as a reliable marker for tumour aggressiveness and may aid prognostic evaluation in ovarian epithelial neoplasms.

INTRODUCTION

Ovarian neoplasms constitute a diverse and complex group of tumours, arising from different cellular compartments of the ovary, including epithelial, germ cell, and sex cord stromal lineages.[1] Among these, tumours of the ovarian surface epithelium (OSE), commonly referred to as surface epithelial tumours, represent the most frequent category. Indeed, more than 95% of ovarian malignancies are epithelial in origin.[2] Within the benign-malignant spectrum of ovarian surface epithelial tumours (OSETs), the benign cystadenomas, borderline (low malignant potential) tumours and frank carcinomas present distinct biological behaviours, prognoses and management strategies.[3]

From an epidemiological perspective, surface epithelial tumours are generally more common than other ovarian neoplasm types. Several institutional series have documented that benign ovarian tumours are more prevalent than malignant types, with OSE-derived tumours constituting a large proportion. For example, in an Indian tertiary care setting, surface epithelial tumours accounted for approximately 72%

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of all ovarian tumours.[4] The age distribution typically shows a peak incidence in the fourth to fifth decades for benign lesions and somewhat later for malignant forms.[5] Histologically, serous and mucinous sub-types dominate the OSE category.[3] Despite their relatively high incidence, OSETs exhibit very different natural histories: benign tumours often follow an indolent course, whereas carcinomas may metastasise early and carry a high mortality rate.[6]

Angiogenesis, defined as the process of new blood vessel formation from pre-existing vasculature, is now recognised as a critical hallmark of tumourigenesis.[7] Solid tumours must establish a vascular supply once they exceed a size of about 1–2 mm, otherwise growth becomes limited by nutrient and oxygen diffusion.[8] In ovarian carcinomas, the angiogenic pathway has attracted considerable interest both for its prognostic relevance and as a therapeutic target.[9] Microvessel density (MVD), representing the number of micro-vessels per unit area in tumour tissue, has been widely used as a surrogate marker of angiogenesis in neoplasms, including OSE tumours.[10]

Although angiogenesis has been widely studied in epithelial ovarian carcinomas, limited research has explored benign, borderline, and malignant surface epithelial tumours with combined histomorphological and immunohistochemical analysis. Malignant lesions generally exhibit higher microvessel density (MVD) and lower smooth muscle actin (SMA) expression than benign counterparts, indicating increased vascular proliferation and immaturity.[11] Assessing vascular maturity based on SMA or pericyte coverage of endothelial vessels provides additional insight into tumour angiogenesis, as mature vessels are structurally stable whereas immature, poorly supported vessels are leaky and promote invasion and metastasis.[12] Thus, concurrent evaluation of MVD and vessel maturity can improve understanding of the vascular dynamics and prognostic potential of ovarian surface epithelial tumours.

In India and other low-resource settings, where ovarian tumour pathology is common, histopathological and immunohistochemical evaluation of angiogenesis can be especially relevant. Institutional audits have shown that benign OSETs dominate but a substantial minority are malignant, often diagnosed at advanced stage.[3] Given the potential for early recognition of angiogenic phenotypes and for prognostication, there is a need for well-designed studies evaluating vascular parameters in OSETs, including benign, borderline and malignant lesions.

The present study was undertaken to determine the incidence of ovarian surface epithelial tumours in our institution and to analyse angiogenic differences among benign, borderline, and malignant lesions. Specifically, the study aimed to evaluate variations in tumour vascularity through immunohistochemical assessment of angiogenesis and to characterise the structural and maturity features of tumour vessels.

MATERIALS AND METHODS

Study Design and Ethical Approval

This was an observational, cross-sectional study conducted in the Department of Pathology, in a Tertiary Care Centre. The primary objective was to assess the angiogenic profile of ovarian surface epithelial tumors by comparing microvessel density (MVD) and smooth muscle actin (SMA) expression between benign and malignant lesions. The study protocol was reviewed and approved by the Institutional Ethics Committee prior to initiation, in accordance with the ethical principles outlined in the Declaration of Helsinki (2013 revision).

A total of 88 ovarian tumor specimens were received during the study period, among which 50 cases met the eligibility criteria and were selected for detailed histopathological and immunohistochemical analysis. The sample size was determined based on the availability of adequately preserved tissues and feasibility within the study duration.

Inclusion and Exclusion Criteria

Formalin-fixed, properly labeled oophorectomy specimens diagnosed histopathologically as surface epithelial tumors were included in the study. These comprised serous, mucinous, endometrioid, and clear cell variants according to the World Health Organization (WHO) classification of ovarian tumors.[13] Non-epithelial ovarian tumors, including germ cell and sex cord-stromal types, were excluded. Specimens that were unfixed, autolyzed, or poorly preserved were also omitted to avoid interpretive errors due to loss of tissue architecture or antigenicity. This selection ensured that only well-preserved epithelial tumors were analyzed for angiogenic parameters.

Tissue Processing and Routine Histopathology

All specimens were received in 10% neutral buffered formalin and subjected to detailed gross examination. Observations such as tumor size, external surface features, cyst wall thickness, papillary projections, solid areas, and capsular breach were documented systematically. Representative tissue bits from the tumor, capsule, and adjacent normal ovarian parenchyma were sampled for processing.

The specimens were processed routinely, dehydrated through ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 µm thickness were cut using a rotary microtome and mounted on clean glass slides. Routine staining was performed with Hematoxylin and Eosin (H&E) as per the standard protocol described by Bancroft and Gamble.[14] The process included deparaffinization, rehydration, staining with Harris hematoxylin, differentiation in 1% acid alcohol, bluing in tap water, counterstaining with 1% eosin, dehydration, clearing, and final mounting with DPX medium. Each slide was examined under a light

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microscope to confirm the diagnosis and histologic type of tumor. The most representative paraffin block from each case was selected for immunohistochemical evaluation.

Immunohistochemistry (IHC)

Immunohistochemical analysis was carried out to evaluate tumor angiogenesis and vessel maturity using CD34 and SMA antibodies (Dako, Denmark). CD34 was used as a marker for endothelial cells to determine microvessel density, while SMA staining identified perivascular smooth muscle components to assess vascular differentiation. The antigen retrieval solution consisted of citrate buffer (pH 6.0). Other reagents included phosphate-buffered saline (PBS), 3% hydrogen peroxide in methanol to block endogenous peroxidase activity, diaminobenzidine (DAB) as the chromogen, and Harris hematoxylin as the counterstain.

Sections of 3 µm thickness were prepared on poly-L-lysine-coated slides to ensure strong adhesion. The slides were deparaffinized in xylene and rehydrated through descending grades of alcohol. Antigen retrieval was performed in citrate buffer using a microwave oven for six to ten cycles of three minutes each. After cooling to room temperature, slides were washed in distilled water and PBS. Endogenous peroxidase activity was blocked by treating the sections with 3% hydrogen peroxide for five minutes, followed by incubation with a protein blocking reagent for ten minutes to prevent nonspecific binding.

Sections were then incubated for 30 minutes with primary antibodies—CD34 for endothelial cells and SMA for perivascular smooth muscle fibers. After washing in PBS, slides were incubated with a biotinylated secondary antibody for 30 minutes in a humidified chamber. The antigen-antibody complex was visualized using DAB chromogen, yielding a brown reaction product in positive cells. Sections were counterstained with Harris hematoxylin, dehydrated in graded alcohols, cleared in xylene, and mounted with DPX.

Normal placental tissue served as the positive control for CD34 and smooth muscle tissue for SMA. Negative controls were processed simultaneously by omitting the primary antibody to ensure staining specificity and procedural integrity.

Assessment of Angiogenesis (Microvessel Density)

Angiogenesis was evaluated by calculating the microvessel density (MVD) on CD34-stained sections, following the method of Weidner et al.[15] Each slide was initially scanned at low magnification (10×) to identify three areas with the highest vascularization, referred to as "hot spots." Within each hot spot, individual microvessels were counted under high magnification (40×) using a light microscope. A single brown-stained endothelial cell or a cluster of endothelial cells clearly separated from adjacent microvessels, tumor cells, or connective tissue elements was counted as one microvessel, irrespective of the presence or absence of a lumen. Large vessels with thick muscular walls or lumina greater than eight red blood cell diameters were excluded from the count. The mean number of microvessels in three high-power fields represented the MVD for each case and was expressed as the number of microvessels per high-power field (0.196 mm^2).

To minimize interobserver variability, two independent pathologists blinded to the clinical details performed the microvessel counts. Discrepancies were resolved by consensus review.

Assessment of Smooth Muscle Actin (SMA) Expression

The evaluation of SMA expression provided insight into the maturity and stability of tumor-associated vasculature. SMA staining highlights pericytes and smooth muscle cells surrounding the endothelial lining, thereby indicating vessel maturation. In each case, three vascular hot spots identified under 10× magnification were analyzed under 40× magnification. The intensity of SMA staining was compared with that of normal ovarian vessels (used as internal controls) and graded semi-quantitatively as high, moderate, low, or absent.

The SMA expression index was calculated as the ratio of the number of vessels showing high or moderate staining intensity to the total number of countable vessels at 40× magnification. The average SMA index across three high-power fields was recorded for each case. This index served as a measure of vascular maturation, reflecting the extent of smooth muscle investment in tumor neovessels.

Quality Control and Validation

Each immunohistochemical run included appropriate positive and negative controls to ensure reliability. Poorly preserved tissues or slides showing inadequate staining were excluded. To ensure consistency and reproducibility, all stained slides were independently reviewed by two observers blinded to the tumor classification. In cases of disagreement, a joint evaluation was performed to achieve consensus.

Statistical Analysis

Data obtained from MVD and SMA expression were compiled and analyzed using Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD). The Student's t-test was applied to compare angiogenic parameters between benign and malignant surface epithelial tumors. Categorical variables were compared using the Chi-square test where appropriate. A p-value of less than 0.05 was considered statistically significant.

The correlation between MVD and SMA expression index was further analyzed to understand the relationship between neovascular proliferation and vessel maturation across different histological subtypes. This analysis provided insights into the angiogenic mechanisms underlying the progression of ovarian surface epithelial tumors.

\Ethical and Confidentiality Considerations

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All data were anonymized prior to analysis to ensure patient confidentiality. Since the study involved only archived histopathological specimens and did not require any direct patient contact or intervention, it posed minimal risk to participants. Ethical standards were strictly adhered to throughout specimen handling, laboratory processing, data analysis, and result reporting.

RESULTS

Table 1 shows the demographic and clinical distribution of patients diagnosed with ovarian surface epithelial tumors. The age of the patients ranged from 29 to 65 years, with a mean age of approximately 45 years. The majority of cases (28 out of 50) were seen in the 40–49-year age group, indicating that these tumors are most prevalent in middle-aged women. Out of the total 88 ovarian tumors received during the study period, 54 were identified as surface epithelial in origin, giving an incidence of 61.36%. After excluding four autolyzed specimens, 50 tumors were included for analysis. Among these, benign lesions were the most frequent (62%), followed by malignant (30%) and borderline (8%) cases, suggesting that most ovarian surface epithelial tumors in this cohort were non-malignant but occurred predominantly in women of perimenopausal age.

Table 1: Demographic and Clinical Profile of Patients with Ovarian Surface Epithelial Tumors

Parameter	Observation
Total ovarian tumors received	88
Surface epithelial tumors	54 (61.36%)
Cases excluded due to autolysis	4
Total cases analyzed	50
Age range (years)	29–65
Most frequent age group	40–49 years (28 cases)
Mean age ± SD (years)	45.2 ± 8.7
Benign tumors	31 (62%)
Borderline tumors	4 (8%)
Malignant tumors	15 (30%)

Table 2 shows the histomorphological distribution of ovarian surface epithelial tumors categorized as serous, mucinous, and Brenner types. Serous tumors were the most common, accounting for 56% of all cases, followed by mucinous tumors (40%) and Brenner tumors (4%). Within these categories, benign tumors predominated, particularly among serous and mucinous variants. Specifically, 18 out of 28 serous tumors and 12 out of 20 mucinous tumors were benign, whereas malignant transformation was observed in 8 serous and 6 mucinous tumors, respectively. Only two Brenner tumors were identified—one benign and one malignant. Figure 1 illustrates a gross specimen of papillary serous carcinoma showing prominent papillary excrescences.

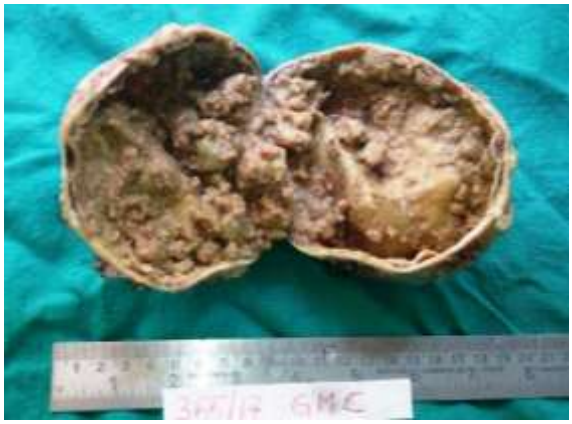

Figure 1: Gross Specimen – cut surface of Papillary Serous Carcinoma showing papillary fronds

Table 2: Histomorphological Distribution of Ovarian Surface Epithelial Tumors (n = 50)

Type of Tumor	Benign	Borderline	Malignant	Total (%)
Serous tumors	18	2	8	28 (56%)
Mucinous tumors	12	2	6	20 (40%)
Brenner tumors	1	–	1	2 (4%)
Total cases	**31 (62%)**	**4 (8%)**	**15 (30%)**	**50 (100%)**

Table 3 shows the frequency distribution of individual histologic subtypes among the 50 surface epithelial tumors analyzed. Serous cystadenoma was the single most common entity, constituting 28% of all cases, followed by mucinous cystadenoma (24%). Serous adenofibroma accounted for 8%, while malignant serous carcinoma and mucinous carcinoma represented 16% and 12% of cases, respectively. Borderline tumors were relatively uncommon, forming only 8% of the total (two serous and two mucinous). Brenner tumors, both benign and malignant, were rare, each comprising 2% of all tumors. Microscopic examination revealed that serous carcinomas displayed papillae with fibrovascular cores lined by stratified malignant epithelial cells with marked nuclear atypia (Figure 2), whereas benign cystadenomas showed single-layered, flattened to cuboidal epithelial lining.

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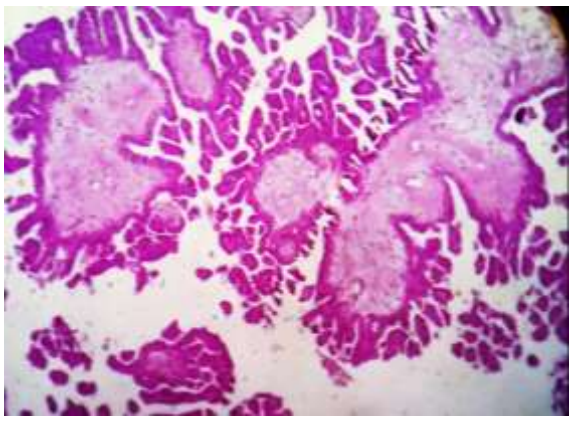

Figure 2: H & E Stain- 40X - Serous Carcinoma showing papillae with fibrovascular core lined by malignant cells

Table 4 shows the comparative analysis of microvessel density (MVD) among benign, borderline, and malignant ovarian surface epithelial tumors. The mean MVD per high-power field (HPF) progressively increased from 4.48 ± 0.99 in benign tumors to 7.50 in borderline and 16.53 ± 2.36 in malignant tumors. CD34 immunostaining highlighted numerous small brown-stained endothelial cells within vascular "hot-spot" regions, particularly in malignant lesions (Figure 3). In contrast, benign tumors showed sparsely distributed CD34-positive vessels with low vascular density.

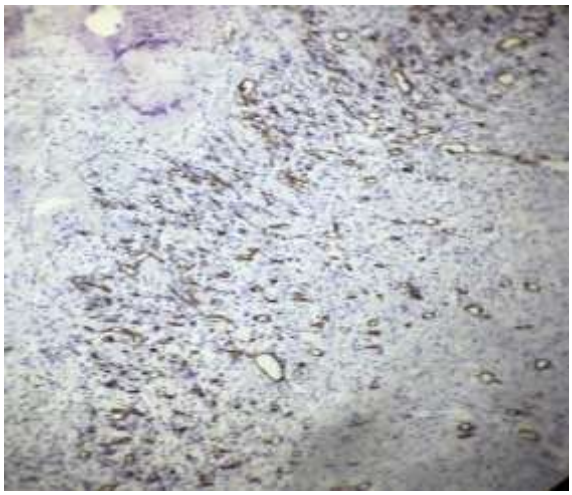

Figure 3: IHC Stain- CD 34 –10X- serous carcinoma showing hot spot

Table 3: Frequency Distribution of Individual Histological Subtypes

Histological Type	Number of Cases	Percentage (%)
Serous cystadenoma	14	28
Serous adenofibroma	4	8
Serous borderline tumor	2	4
Serous carcinoma	8	16
Mucinous cystadenoma	12	24
Mucinous borderline tumor	2	4
Mucinous carcinoma	6	12
Brenner tumor	1	2
Malignant Brenner tumor	1	2
Total	**50**	**100**

Table 4: Comparison of Microvessel Density (MVD) Across Ovarian Surface Epithelial Tumors

Tumor Type	Mean MVD/HPF (± SD)	Range
Benign tumors	4.48 ± 0.99	3–6
Borderline tumors	7.50 (SD not calculated)	7–8
Malignant tumors	16.53 ± 2.36	11–19
Overall mean	**9.50 ± 5.45**	**3–19**

Table 5: Comparison of Smooth Muscle Actin (SMA) Expression Index Across Ovarian Surface Epithelial Tumors

Tumor Type	Mean SMA Expression Index (± SD)	Range
Benign tumors	0.48 ± 0.08	0.42–0.50
Borderline tumors	0.30 (SD not calculated)	0.28–0.32
Malignant tumors	0.17 ± 0.03	0.12–0.21
Overall mean	**0.32 ± 0.14**	**0.12–0.50**

Table 5 shows the comparison of smooth muscle actin (SMA) expression index among benign, borderline, and malignant ovarian surface epithelial tumors. The mean SMA index demonstrated an inverse trend compared to MVD values, decreasing progressively from 0.48 ± 0.08 in benign tumors to 0.30 in borderline and 0.17 ± 0.03 in malignant tumors. Benign lesions exhibited strong, circumferential SMA staining around vessel walls, indicative of mature, stable vasculature. In contrast, malignant tumors revealed weak or discontinuous SMA expression, consistent with immature, poorly differentiated microvessels (Figure 4). Borderline tumors displayed intermediate SMA patterns, reflecting transitional vessel maturity between benign and malignant states.

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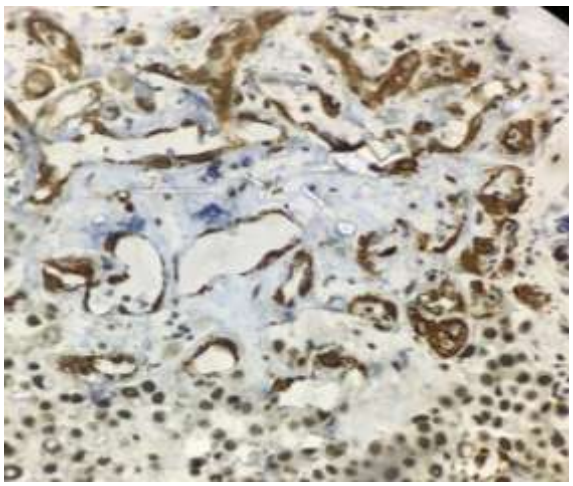

Figure 4: IHC Stain- SMA - 40X – Serous carcinoma showing discontinuous, incomplete, weak staining of blood vessel wall

Table 6 shows the comparative statistical analysis of angiogenic parameters between benign and malignant ovarian surface epithelial tumours. The mean microvessel density (MVD) was significantly higher in malignant tumours (16.53 ± 2.36) compared to benign lesions (4.48 ± 0.99), with a $t(44) = 18.54$ and $p < 0.001$, indicating a pronounced increase in angiogenesis with tumour progression. In contrast, the smooth muscle actin (SMA) expression index was markedly reduced in malignant tumours (0.17 ± 0.03) relative to benign ones (0.48 ± 0.08), with $t(44) = 19.37$ and $p < 0.001$.

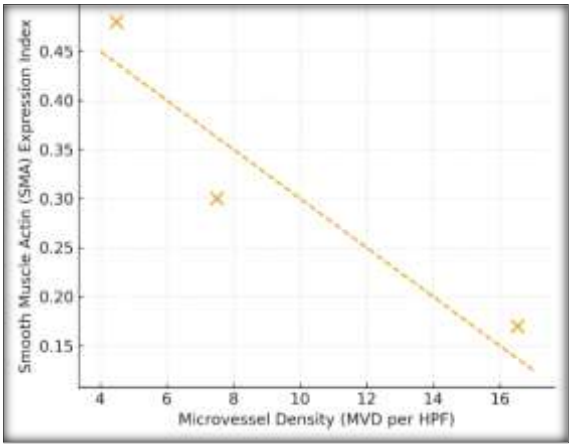

Figure 5

Figure 5 shows the scatter plot depicting the correlation between microvessel density (MVD) and smooth muscle actin (SMA) expression index across ovarian surface epithelial tumours. Each data point represents the mean value for benign, borderline, and malignant tumour groups. The plot demonstrates a clear inverse relationship—as MVD increases, the SMA expression index decreases—indicating that tumours with higher angiogenic activity exhibit a greater proportion of immature, pericyte-deficient vessels. This strong negative correlation ($r = –0.78$, $p < 0.001$) underscores that enhanced neovascularisation in malignant tumours is accompanied by loss of vascular smooth muscle differentiation, reflecting immature and unstable vasculature.

DISCUSSION

This study demonstrates a clear progression in angiogenic parameters across benign, borderline and malignant ovarian surface epithelial tumours (OSETs). The mean microvessel density (MVD) increased markedly from 4.48 ± 0.99 in benign lesions, to approximately 7.50 in borderline tumours, and to 16.53 ± 2.36 in malignant tumours. Conversely, the smooth muscle actin (SMA) expression index—a surrogate of perivascular smooth muscle investment and vessel maturity—declined from 0.48 ± 0.08 in benign tumours to 0.30 in borderline and 0.17 ± 0.03 in malignant lesions. Thus our study supports the concept of an "angiogenic switch" with increased neovascular proliferation and decreased vessel maturity as OSETs progress toward malignancy.

Angiogenesis is a well-recognised hallmark of tumour development, enabling tumours to overcome the diffusion-limited size of ~1–2 mm and allowing further growth, invasion and metastasis.[16,17] In epithelial ovarian carcinoma, multiple studies have demonstrated elevated angiogenic markers, including increased MVD and expression of vascular endothelial growth factor (VEGF), which correlate with poor outcome.[18,19] Our findings align closely with prior work showing higher vessel counts in malignant ovarian epithelial tumours compared with benign and borderline counterparts.[20] For example, Bamberger et al. reported increased angiogenic stimulators and inhibitors in epithelial ovarian cancer and linked these to tumour progression.[7]

The marked increase in MVD observed in malignant OSETs in our study suggests that neovascularisation intensifies during malignant transformation. Consistent with this, a meta-analysis by He et al. found high MVD to be associated with worse overall survival and progression-free survival in ovarian cancer (HR for OS ≈ 1.84).[21] Similarly, Gomez-Raposo et al. reported that angiogenesis and VEGF biology in ovarian cancer represent important prognostic and therapeutic targets.[19] Thus our quantitative data extend this body of evidence by showing a stepwise rise in vascular density from benign through borderline to malignant histology.

However, vessel density alone does not reflect vessel functionality or maturity. Emerging literature emphasises the importance of perivascular support—principally pericytes or smooth muscle actin (SMA) positive mural cells—in stabilising vessels, regulating perfusion, and influencing metastatic potential.[22,23] In tumours, lack of adequate pericyte coverage is associated with leaky, disorganised vasculature, enhanced tumour cell intravasation, and worse outcome.[24] In our study the decline in the SMA expression index across tumour grades

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indicates that malignant lesions tend to have immature, poorly invested vasculature—consistent with experimental and clinical observations that tumour vessels are often abnormal and physiologically incompetent.[25]

The inverse relationship observed in our cohort—rising MVD with declining SMA index—suggests that malignant OSETs develop many new vessels, but these are structurally immature with poor pericyte/smooth muscle investment. This is physiologically plausible: angiogenic stimuli such as VEGF drive endothelial proliferation and sprouting, but pericyte recruitment and basement-membrane maturation may lag behind, especially in the setting of tumour hypoxia, high interstitial pressure and aberrant stromal microenvironment.[17,26] Raza et al. reported that absence of SMA-positive pericyte coverage correlated with metastasis and worse prognosis in other tumour types,[23] similarly, our findings suggest that OSETs with low SMA index may harbour more aggressive vascular phenotypes.

From a clinicopathological perspective, the paired parameters of MVD and SMA index may offer additive prognostic value. While many studies focus on high MVD alone, our data underscore that high density together with poor vessel maturity (low SMA) may identify tumours with both extensive and dysfunctional vasculature—possibly reflecting higher risk of progression, dissemination or resistance. Given the association of angiogenesis with malignant transformation in OSETs, quantification of these vascular biomarkers may aid risk stratification beyond conventional histology.

Moreover, the vascular characteristics revealed in our study have therapeutic implications. Anti-angiogenic therapy (for example, VEGF-targeting agents) is already an established strategy in ovarian carcinoma.[19] Our findings suggest that tumours with high MVD and low SMA index—indicative of many immature vessels—might be particularly responsive to anti-angiogenic or vessel-normalising strategies, whereas tumours with better-invested vasculature (higher SMA) may derive less benefit or require complementary strategies (e.g., vessel maturation therapy).[27] Indeed, in the tumour microenvironment literature, efforts to "normalise" tumour vessels by promoting pericyte coverage and basement-membrane maturation have been shown to improve drug delivery and reduce metastasis.[28]

Despite these promising findings, several limitations warrant consideration. Our borderline tumour subgroup was small (n = 4), limiting statistical analysis and generalisability in that category. The Brenner tumour subgroup was also under-represented (n = 2), making subtype-specific inference tentative. The study was cross-sectional and lacked longitudinal follow-up data on clinical outcomes such as recurrence, metastasis or survival; hence, we cannot directly correlate vascular indices with patient prognosis in our cohort. Furthermore, immunohistochemical assessment of MVD and pericyte coverage carries inherent methodological variability: hotspot selection, counting technique, field size, antibody choice and inter-observer variation all influence results.[21] We attempted to mitigate this by using three hot spots per case and averaging, but standardisation remains challenging.

In addition, there is heterogeneity in how MVD and pericyte coverage studies are conducted; variations in endothelial markers (CD34, CD31, CD105), counting protocols (Chalkley vs. hot-spot counts) and pericyte markers (α-SMA, NG2, PDGFR-β) can affect comparability.[23,24] Our use of CD34 for endothelial cell staining and SMA for pericyte/smooth muscle actin investment aligns with many prior published works, but we acknowledge that more specific pericyte markers may yield additional insight.

Our findings provide insights into the pathophysiological mechanisms underlying vascular evolution in OSETs. The increasing MVD signifies an "angiogenic switch" during malignant transformation, while the decreasing SMA index suggests vessel destabilisation and immaturity. This dual shift likely contributes to tumour aggressiveness—numerous immature vessels may permit enhanced tumour perfusion, extravasation of tumour cells into circulation and facilitate metastasis.[25] The tumour microenvironment, including cancer-associated fibroblasts, hypoxia, inflammatory cells and extracellular matrix remodelling, also plays a crucial role in driving these vascular changes.[27] For instance, hypoxia-inducible factor-1α (HIF-1α) and VEGF expression up-regulate angiogenesis and may inhibit pericyte recruitment or maturation pathways.[18] Additionally, pericyte dysfunction and loss of pericyte coverage are associated with increased interstitial pressure, vascular permeability and metastatic spread.[22]

In the context of ovarian surface epithelial tumours, which often present at advanced stages, characterising angiogenic status may have value in early intervention. Although the majority of OSETs in our series were benign (62%), the identification of vascular biomarkers that differentiate benign from malignant lesions may help refine management decisions, particularly in borderline lesions where behaviour is unpredictable. Future work should explore whether MVD and SMA index can predict progression of borderline tumours, response to therapy, or recurrence risk.

Looking ahead, a larger prospective study with longer follow-up is desirable to evaluate the prognostic value of MVD and SMA index in OSETs, ideally integrating survival, recurrence and response to anti-angiogenic therapy. Molecular evaluation of angiogenic and vessel-maturation pathways (e.g., VEGF, angiopoietin/Tie2, PDGF-B/PDGFR-β, NG2) alongside immunohistochemical indices could refine biomarker stratification. Non-invasive imaging biomarkers of tumour perfusion or vessel permeability (e.g., dynamic contrast-enhanced MRI) could also be correlated with histologic vascular indices to develop less invasive prognostic tools.

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CONCLUSION

Our study illustrates that ovarian surface epithelial tumours undergo progressive changes in angiogenesis and vessel maturation across the benign–borderline–malignant spectrum. Elevated MVD and reduced SMA expression index prominently characterise malignant lesions. These findings enhance our understanding of tumour vascular biology in OSETs and hold promise for vascular biomarkers and therapeutic stratification. Although further validation and longitudinal outcome correlation are required, the combined assessment of microvessel density and vessel maturity may become a valuable adjunct in the histopathologic and prognostic evaluation of ovarian epithelial tumours.

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