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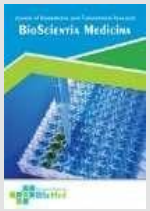

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Unraveling the Angiogenic Landscape in Endometrioid Endometrial Carcinoma: VEGF Expression, Histopathological Differentiation, and Lymphovascular Invasion as Key Players

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A B S T R A C T

Background: Endometrioid endometrial carcinoma (EEC) is a prevalent gynecological malignancy whose prognosis is influenced by factors including histopathological grade and lymphovascular invasion (LVI). Angiogenesis, crucial for tumor growth and metastasis, is significantly mediated by vascular endothelial growth factor (VEGF). This study aimed to investigate the expression of VEGF in EEC and its correlation with histopathological differentiation and LVI. **Methods:** This observational analytical study employed a cross-sectional design using 36 archival paraffin block samples of EEC diagnosed between January 2022 and December 2024 at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital Padang. Cases were selected via simple random sampling from a population of 59. Histopathological grade (Grade 1, 2, or 3 based on FIGO architectural and nuclear criteria) and LVI (negative, focal, or substantial) were re-evaluated from Hematoxylin-Eosin (H&E) stained slides. VEGF expression was assessed by immunohistochemistry, scored semiquantitatively based on the percentage of positive tumor cells and staining intensity, and categorized as low or high. Data were analyzed using Chi-square tests, with $p<0.05$ considered statistically significant. **Results:** The mean age of patients was 54.36 years, with the highest prevalence in the 51-60 age group (41.7%). Grade 3 tumors were most common (38.9%), followed by Grade 2 (33.3%) and Grade 1 (27.8%). LVI was present in 47.2% of cases, predominantly focal (38.9%). High VEGF expression was observed in 58.3% of EEC cases. A statistically significant association was found between high VEGF expression and higher histopathological grade ($p=0.000$), with 66.7% of Grade 3 tumors showing high VEGF expression. No significant association was found between VEGF expression and LVI ($p=0.080$). **Conclusion:** High VEGF expression significantly correlated with higher histopathological grades in EEC, suggesting its role in tumor aggressiveness and dedifferentiation. However, a significant association with LVI was not established in this cohort. VEGF expression warrants further investigation as a potential prognostic biomarker and therapeutic target in EEC.

1. Introduction

Endometrial carcinoma (EC) stands as one of the most frequently diagnosed gynecological malignancies globally, particularly in developed nations, and its incidence and mortality rates have demonstrated a concerning upward trend in recent years. Global Cancer Observatory (GLOBOCAN) data from 2022 indicated 420,242 new cases and 97,370 deaths worldwide due to endometrial cancer. In Indonesia, GLOBOCAN 2020 reported approximately 7,773 new

cases and 2,626 deaths, with a five-year prevalence of 22,087 cases. This malignancy predominantly affects peri- and postmenopausal women, with the peak incidence occurring between the fifth and sixth decades of life, although a smaller percentage of cases (less than 5%) are observed in women under 40 years of age.[1,2]

Endometrial carcinoma is a heterogeneous group of tumors originating from the epithelial cells of the endometrial glands. It is broadly classified into Type I and Type II based on distinct clinicopathological characteristics, immunohistochemical profiles, and molecular alterations. Type I EC, which accounts for the majority of cases (70-80%), is typically estrogen-dependent and includes endometrioid endometrial carcinoma (EEC) of low to intermediate grade (FIGO grades 1 and 2). These tumors are often preceded by endometrial hyperplasia, tend to be slow-growing, and generally have a more favorable prognosis. Molecularly, Type I tumors are frequently characterized by mutations in genes such as PTEN, KRAS, and CTNNB1 (β-catenin), as well as microsatellite instability (MSI). In contrast, Type II EC is estrogen-independent, encompasses high-grade EEC (FIGO grade 3) and non-endometrioid histologies like serous and clear cell carcinomas, and is often associated with TP53 mutations and HER2/neu overexpression. These tumors are generally more aggressive and carry a poorer prognosis.[3,4]

Endometrioid endometrial carcinoma (EEC) is the most common histological subtype, representing approximately 70-80% of all endometrial cancers. While EEC generally has a better prognosis compared to non-endometrioid types, with a 5-year overall survival rate reported around 88%, recurrence occurs in about 13-17% of patients, typically within three years of primary treatment, significantly worsening outcomes. Accurate risk stratification is crucial to guide therapeutic decisions and avoid under- or over-treatment. Key prognostic factors currently utilized for risk stratification include the FIGO stage, depth of myometrial invasion, histopathological grade, and the presence of lymphovascular invasion (LVI).[5,6]

Histopathological grading in EEC, typically a three-tiered system (Grade 1: well-differentiated, Grade 2: moderately differentiated, and Grade 3: poorly differentiated), is a cornerstone in prognostic assessment and clinical decision-making. The grading is based on the architectural pattern (percentage of solid growth) and nuclear atypia. Higher tumor grade is strongly associated with increased rates of recurrence and decreased survival. For instance, Grade 3 tumors have been shown to have a more than threefold increased risk of recurrence compared to Grade 1 tumors.

Lymphovascular invasion (LVI), defined as the presence of tumor cells within endothelial-lined lymphatic or blood vascular channels, has gained increasing recognition as an independent adverse prognostic factor in EEC, particularly in early-stage disease. LVI is associated with an increased risk of lymph node metastasis, distant recurrence, and reduced overall survival. The extent of LVI (focal versus substantial/extensive) further refines its prognostic value, with substantial LVI being a particularly strong predictor of poor outcomes. The significance of LVI is underscored by its inclusion as a staging factor in the updated FIGO 2023 staging system for endometrial cancer, where substantial LVI can upstage a tumor confined to the uterine corpus.[7,8]

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a fundamental process in tumor growth, progression, and metastasis. Tumors require an adequate blood supply to exceed a minimal size (1-2 mm^3) and to facilitate the dissemination of cancer cells. This process is tightly regulated by a balance of pro-angiogenic and anti-angiogenic factors. When this balance shifts towards pro-angiogenic stimuli, an "angiogenic switch" occurs, promoting neovascularization. Vascular endothelial growth factor (VEGF), particularly VEGF-A, is a potent and critical pro-angiogenic cytokine that plays a pivotal role in stimulating endothelial cell proliferation, migration, survival, and vascular permeability. VEGF exerts its effects by binding to its receptors (VEGFR-1, VEGFR-2, and VEGFR-3) on

endothelial cells, initiating downstream signaling pathways that drive angiogenesis.

VEGF expression has been reported to be elevated in various cancers, including endometrial carcinoma, and is often correlated with tumor aggressiveness, advanced stage, higher grade, myometrial invasion, LVI, lymph node metastasis, and poorer patient outcomes. Studies have shown that increased VEGF levels, both in serum and tumor tissue, can serve as an indicator of tumor angiogenesis and may predict an unfavorable prognosis in EEC patients. Molecular changes, such as increased VEGF expression, often precede phenotypic alterations in EEC, suggesting that early detection of VEGF could aid in timelier diagnosis and more tailored therapeutic strategies. The integration of VEGF as a biomarker alongside established histopathological prognostic factors could enhance risk stratification and facilitate more individualized treatment approaches, potentially improving survival rates.

However, the literature presents some conflicting findings regarding the precise relationship between VEGF expression and specific clinicopathological parameters in EEC, including histopathological grade and LVI. Some studies have reported a strong correlation between high VEGF expression and high-grade tumors or advanced stages, while others have found no significant association with tumor grade, depth of myometrial invasion, or LVI. These discrepancies may arise from variations in study design, sample size, patient populations, immunohistochemical methodologies (including antibody selection and scoring criteria), and diagnostic criteria for histopathological features. The interpretation of VEGF staining can be influenced by technical factors such as the duration of tissue block storage, which might affect antigenicity and staining quality. Furthermore, VEGF expression is not limited to tumor cells; it can also be found in stromal components like cancer-associated fibroblasts (CAFs), which are influenced by the tumor microenvironment, including hypoxia and cytokine signaling (IL-6), further complicating the interpretation of its overall role.

The novelty of this research resided in its focused investigation of VEGF expression in correlation with detailed histopathological grading and LVI status within a cohort of EEC patients from West Sumatra, Indonesia. While VEGF's role in cancer angiogenesis is broadly acknowledged, its precise correlative significance with these specific prognostic indicators in EEC has yielded variable results across different populations and study designs. This study aimed to contribute to clarifying these relationships by employing a standardized re-evaluation of histopathology and a semiquantitative assessment of VEGF expression on archival tissues from a defined regional medical center. Such regional data are crucial for validating biomarkers and understanding disease patterns that might inform local clinical practice. Furthermore, this study sought to provide foundational data on VEGF expression patterns in EEC within this specific Indonesian population, where such detailed correlative studies have been scarce.[9,10] Therefore, the primary aim of this study was to analyze the relationship between vascular endothelial growth factor (VEGF) expression and two key prognostic factors—histopathological grade and lymphovascular invasion—in endometrioid endometrial carcinoma cases diagnosed at Dr. M. Djamil General Hospital Padang.

2. Methods

This study employed an observational analytical approach with a cross-sectional design. The research was conducted to investigate the relationship between vascular endothelial growth factor (VEGF) expression and key clinicopathological parameters, specifically histopathological grade and lymphovascular invasion (LVI), in endometrioid endometrial carcinoma (EEC). Data collection and laboratory work were performed at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital, Padang, West Sumatra, Indonesia. The study protocol received ethical clearance and approval from the Research Ethics Committee of the

Faculty of Medicine, Andalas University, and the Research Ethics Committee of Dr. M. Djamil General Hospital Padang. As the study utilized archived, anonymized biological materials (slides and paraffin blocks) and involved no direct patient contact or intervention, individual patient consent was deemed unnecessary, with patient confidentiality strictly maintained throughout the research process. The target population consisted of all cases diagnosed histopathologically as EEC at the study institution between January 2022 and December 2024, totaling 59 cases identified from laboratory records. Samples for the study were selected from this population using a simple random sampling technique. The inclusion criteria were: histopathologically confirmed EEC diagnosis; availability of complete medical record data; history of total hysterectomy as primary surgical management; and availability of adequate quality formalin-fixed, paraffin-embedded (FFPE) tissue blocks suitable for histological re-evaluation and immunohistochemistry. No specific exclusion criteria were applied beyond failure to meet the inclusion criteria. The required sample size was calculated based on an anticipated proportion (P) of VEGF expression in EEC of 88%, with a desired precision (d) of 10% (0.10) and a 95% confidence level ($Z1-\alpha/2 = 1.96$), using the formula for finite populations (N=59). This yielded a minimum required sample size of 25 cases, which was increased by approximately 10% to account for potential exclusions, resulting in a target sample size of 28. A total of 36 EEC cases ultimately met the inclusion criteria and were included in the final analysis. Clinicopathological data, including patient age at diagnosis, were extracted from the corresponding medical records. Age was categorized for analysis into groups (≤50 years, 51-60 years, 61-70 years). The primary study variables were defined as follows: Independent Variable**:** VEGF expression level in tumor tissue; Dependent Variables: Histopathological grade and lymphovascular invasion (LVI) status.

Archival Hematoxylin and Eosin (H&E) stained slides corresponding to the selected cases were retrieved. In instances where original slides were missing or suboptimal for assessment, new 3µm sections were cut from the FFPE blocks using a microtome, mounted on glass slides, and stained with H&E following standard laboratory protocols. All H&E slides underwent re-evaluation by the primary researcher and were confirmed by two senior anatomical pathologists (supervisors) to verify the EEC diagnosis and assess the dependent variables using an Olympus CX-23 binocular light microscope (Olympus Corporation, Tokyo, Japan) at magnifications ranging from 40x to 400x. Tumor grading was performed according to the 2014/2020 FIGO/WHO classification system, integrating architectural patterns and nuclear features. The architectural grade was based on the percentage of solid (non-squamous, non-morular) growth within the tumor: Grade 1 (≤5%), Grade 2 (6-50%), Grade 3 (>50%). Nuclear grading assessed atypia based on variation in nuclear size and shape, chromatin distribution, and nucleolar prominence. If notable nuclear atypia (Grade 3 features: marked pleomorphism, vesicular or coarse chromatin, prominent nucleoli) was identified in an architecturally Grade 1 or 2 tumor, the overall grade was elevated by one level. The final grade was recorded as Grade 1, 2, or 3 (Ordinal scale).

Lymphovascular Invasion (LVI) was defined as the unequivocal presence of tumor cells within an endothelial-lined vascular or lymphatic space within the tumor stroma or myometrium. Morphological criteria included identifying tumor cell clusters conforming to vascular spaces, adherence to vessel walls, presence within spaces lined by flattened endothelial cells, and association with hematopoietic elements or adjacent thick-walled vessels. LVI assessment was performed solely on H&E sections and reported semiquantitatively as: Negative: Absence of LVI; Positive - Focal: Involvement of ≤3 vascular spaces in a single representative slide; Positive - Substantial/Extensive: Involvement of ≥4 vascular spaces in at least one slide. The final LVI status was recorded using an ordinal scale (Negative, Positive

Focal, Positive Substantial). The study acknowledged the limitation of not using ancillary IHC markers (CD31, D2-40) for LVI confirmation.

Immunohistochemical staining for VEGF was performed on 3 µm-thick sections cut from the selected FFPE blocks and mounted on silane-coated glass slides. The manual staining procedure employed the Streptavidin-Biotin Complex (SBC) detection method and involved the following key steps: Drying at 37°C followed by heating on a slide warmer at 60°C; Xylene washes (3 x 5 min) followed by graded alcohols (100% to 70%, 5 min each) and rinsing in running water; Heat-induced epitope retrieval (HIER) using 10 mM Sodium Citrate buffer (pH 6.0) in a microwave oven at 95°C for 15 minutes, followed by cooling; Incubation in 0.3% hydrogen peroxide (H_2O_2) in PBS for 30 minutes at room temperature; Incubation with 1.5% normal blocking serum for 30 minutes; Application of the primary antibody against VEGF (source/clone not specified, diluted 1:40) and incubation for 1 hour at room temperature in a humidified chamber; Application of a universal link secondary antibody (Trekkie Universal Link) for 30 minutes at room temperature; Application of TrekAvidin-HRP complex for 30 minutes at room temperature; Visualization using 3,3'-Diaminobenzidine (DAB) chromogen solution for 5 minutes; Staining with Modified Mayer's Hematoxylin for 5 minutes; Dehydration through graded alcohols, clearing in xylene (5 min), and permanent mounting with coverslips using Entellan mounting medium; All incubation steps were followed by appropriate rinsing steps using PBS buffer. VEGF expression was assessed based on cytoplasmic staining within the tumor cells, observed under the Olympus CX23 light microscope (40x, 100x, 200x magnification). A semiquantitative scoring system, combining the proportion of positive cells and staining intensity, was utilized, reportedly aided by ImageJ software: Proportion Score: Based on the percentage of positively stained tumor cells (averaged over ten 40x fields): Score 0 (0%), Score 1 (1% to <25%), Score 2 (25% to 49%), Score 3 (≥50%); Intensity Score: Based on the average staining intensity: Score 0 (Negative), Score 1 (Weak), Score 2 (Moderate), Score 3 (Strong). An Immunoreactive Score (IRS) was calculated by summing the Proportion Score and the Intensity Score (range 0-6). For statistical analysis, the IRS was dichotomized into two categories: Low Expression: IRS of 0-2 (Negative or Weak); High Expression**:** IRS of 3-6 (Moderate or Strong). The scoring was performed by the primary researcher and validated by the supervising pathologists.

Data collected were entered and analyzed using appropriate statistical software, SPSS version 27. Descriptive statistics (frequencies, percentages, means, ranges) were calculated to summarize the clinicopathological characteristics (age, grade, LVI) and VEGF expression levels. Results were presented in tables and narrative text. The association between the categorical variables of VEGF expression (Low/High) and histopathological grade (Grade 1/2/3), and between VEGF expression and LVI status (Negative/Positive), was assessed using the Pearson Chi-Square ($\chi 2$) test or Fisher's Exact test where appropriate (if expected cell counts were lower than 5). A p-value less than 0.05 ($p<0.05$) was considered statistically significant.

3. Results

Table 1 showed a comprehensive overview of the clinicopathological characteristics of the 36 patients diagnosed with endometrioid endometrial carcinoma (EEC) included in this study, offering valuable insights into the demographic and tumor-specific features within this cohort. Table 1 showed that the age of the patients at diagnosis ranged from a relatively young 28 years to an older 70 years, with a calculated mean age of 54.36 years. This means age aligns with the general understanding that EEC predominantly affects peri- and postmenopausal women, although the presence of a 28-year-old patient underscores that this malignancy can, albeit less commonly, manifest in younger individuals. When categorized into age groups, the highest prevalence of EEC was observed in patients aged 51-60 years, accounting for 41.7% (15

out of 36 cases) of the study population. Patients aged ≤50 years constituted 30.6% (11 cases), indicating a significant proportion of cases occurring before the typical postmenopausal period, while the 61-70 years age group represented 27.8% (10 cases). This age distribution highlights the necessity for vigilance and diagnostic consideration of EEC across a broad spectrum of adult female life, particularly from middle age onwards. Table 1 showed that concerning the tumor's histological differentiation, as per the FIGO grading system, there was a notable distribution across the grades, with a tendency towards higher-grade tumors in this particular cohort. Poorly differentiated (Grade 3) carcinomas were the most frequent, identified in 38.9% (14 out of 36) of cases. Moderately differentiated (Grade 2) tumors followed, comprising 33.3% (12 cases), while well-differentiated (Grade 1) carcinomas were found in 27.8% (10 cases). This distribution, particularly the higher proportion of Grade 3 tumors, is significant as histopathological grade is a critical prognostic factor in EEC, with higher grades generally correlating with more aggressive tumor behavior, increased risk of recurrence, and poorer patient outcomes. The predominance of Grade 3 tumors might reflect the referral patterns to the institution where the study was conducted or could indicate a genuinely more aggressive tumor profile in the sampled population. Table 1 showed that Lymphovascular invasion, a key indicator of tumor invasiveness and metastatic potential, was present in nearly half of the cases, specifically in 47.2% (17 out of 36 patients). Among these 17 LVI-positive cases, focal LVI was the predominant pattern, observed in 14 instances. This accounts for 82.4% of all LVI-positive cases and 38.9% of the total study population. Substantial or extensive LVI, which often carries a more ominous prognosis, was less common, identified in 3 of the LVI-positive cases, representing 17.6% of LVI-positive tumors and 8.3% of the entire cohort. The remaining 52.8% (19 cases) were negative for LVI. The notable presence of LVI, especially focal LVI, underscores the invasive potential within this group of EECs and is a critical parameter for risk stratification and adjuvant treatment decisions. Table 1 showed that the expression of vascular endothelial growth factor (VEGF), a key mediator of angiogenesis, was also assessed. High VEGF expression was detected in the majority of the tumors, specifically in 58.3% (21 out of 36 cases). Conversely, low VEGF expression was observed in 41.7% (15 cases). The predominance of high VEGF expression suggests that angiogenesis is likely an active and significant biological process in a substantial portion of these EECs, potentially contributing to tumor growth, progression, and the establishment of a supportive tumor microenvironment. This finding sets the stage for further analysis of how VEGF expression correlates with other aggressive features like tumor grade and LVI.

Table 2 showed a compelling and statistically significant relationship between the expression levels of vascular endothelial growth factor (VEGF) and the degree of histopathological differentiation in the 36 cases of endometrioid endometrial carcinoma (EEC) analyzed (Pearson χ^2= 19.954, p=0.000). This table provides critical quantitative data illuminating how VEGF, a key angiogenic factor, correlates with tumor grade, a fundamental prognostic indicator in EEC. An examination of the 15 tumors characterized by low VEGF expression revealed a striking skew towards better differentiation. The vast majority of these cases, 60.0% (9 out of 15), were classified as Grade 1 (well-differentiated). Furthermore, 40.0% (6 out of 15) were Grade 2 (moderately differentiated). Most significantly, not a single case (0.0%) exhibiting low VEGF expression was found to be Grade 3 (poorly differentiated). This pattern strongly suggests that EEC tumors with limited VEGF expression are predominantly those with lower histological grades, implying potentially lower angiogenic dependency and possibly a less aggressive biological phenotype. In sharp contrast, the 21 tumors demonstrating high VEGF expression were predominantly associated with poor differentiation. An overwhelming 66.7% (14 out of 21) of these high-VEGF cases were classified as Grade 3. While Grade 2 tumors constituted 28.6% (6

out of 21) of this group, only a very small fraction, 4.8% (1 out of 21), were Grade 1. This distribution underscores a powerful association between elevated VEGF levels and histological features indicative of increased malignancy, namely poor differentiation. It points towards a scenario where heightened angiogenic signaling, driven by VEGF, is a characteristic feature of high-grade, more aggressive EEC. The extremely low p-value (0.000) derived from the Chi-Square analysis provides robust statistical evidence that the observed association between VEGF expression levels and histopathological grade is not a result of random chance. It confirms a significant trend: as the histopathological grade of EEC increases (indicating poorer differentiation and higher aggressiveness), the likelihood of detecting high VEGF expression also significantly increases.

Table 3 shows the association between the level of vascular endothelial growth factor (VEGF) expression and the status of lymphovascular invasion (LVI) in the 36 analyzed cases of endometrioid endometrial carcinoma (EEC). Among the 15 tumors with low VEGF expression, a majority, 73.3% (11 cases), were found to be negative for LVI. Correspondingly, only 26.7% (4 cases) with low VEGF expression demonstrated positive LVI. This indicates that tumors with lower VEGF expression were less likely to exhibit invasion into lymphatic or vascular channels in this cohort. Conversely, in the 21 tumors exhibiting high VEGF expression, a higher proportion showed evidence of LVI. Specifically, 61.9% (13 cases) with high VEGF were LVI-positive, while 38.1% (8 cases) were LVI-negative. This pattern suggests a trend where tumors expressing higher levels of VEGF were more frequently associated with the presence of LVI compared to those with low VEGF expression. Despite the observed trend showing a higher frequency of LVI in the high VEGF group, the statistical analysis using the Chi-Square test with Continuity Correction yielded a p-value of 0.080. Based on the conventional significance threshold of $p < 0.05$, this result indicates that the association between VEGF expression levels and the presence of LVI was not statistically significant in this particular study population. While the Pearson Chi-Square test returned a potentially significant p-value (0.037), the conclusion of non-significance in the original study was based on the corrected value.

Table 1. Clinicopathological characteristics of endometrioid endometrial carcinoma patients (N=36).

Characteristic	Sub-Category / Statistic	Frequency (n)	Percentage (%)
Patient age (Years)	Mean Age	54.36 years	-
	Age Range (Min - Max)	28 - 70 years	-
	Age Groups:		
	≤50 years	11	30.6%
	51 - 60 years	15	41.7%
	61 - 70 years	10	27.8%
Histopathological grade (FIGO)	Grade 1 (Well-differentiated)	10	27.8%
	Grade 2 (Moderately differentiated)	12	33.3%
	Grade 3 (Poorly differentiated)	14	38.9%
Lymphovascular invasion (LVI)	**Overall Status:**		
	Negative for LVI	19	52.8%
	Positive for LVI	17	47.2%
	If LVI Positive (n=17):		
	Focal LVI	14	82.4% of LVI positive cases (38.9% of total)
	Substantial/Extensive LVI	3	17.6% of LVI positive cases (8.3% of total)
VEGF expression level	Low Expression	15	41.7%
	High Expression	21	58.3%
Total sample size		**36**	**100.0%**

Table 2. Relationship between vascular endothelial growth factor (VEGF) expression and histopathological grade in endometrioid endometrial carcinoma (N=36).

VEGF expression level	Histopathological Grade (FIGO) - Grade 1 (Well-differentiated) n (%)	Histopathological Grade (FIGO) - Grade 2 (Moderately differentiated) n (%)	Histopathological Grade (FIGO) - Grade 3 (Poorly differentiated) n (%)	Total (N)	Percentage (%) within VEGF expression category
Low VEGF expression	9 (60.0%)	6 (40.0%)	0 (0.0%)	**15**	**100.0%**
High VEGF expression	1 (4.8%)	6 (28.6%)	14 (66.7%)	**21**	**100.0%**
Total (N)	**10**	**12**	**14**	**36**	
Percentage (%) of total cases	**27.8%**	**33.3%**	**38.9%**		**100.0%**
Chi-square p-value	**0.000**				

Table 3. Relationship between vascular endothelial growth factor (VEGF) expression and lymphovascular invasion (LVI) status in endometrioid endometrial carcinoma (N=36).

VEGF expression level	Lymphovascular invasion (LVI) - Negative n (%)	Lymphovascular invasion (LVI) - Positive n (%)	Total (N)	Percentage (%) within VEGF expression category
Low VEGF Expression	11 (73.3%)	4 (26.7%)	**15**	**100.0%**
High VEGF Expression	8 (38.1%)	13 (61.9%)	**21**	**100.0%**
Total (N)	**19**	**17**	**36**	
Percentage (%) of Total Cases	**52.8%**	**47.2%**		**100.0%**
Chi-Square p-value	**0.080***			
(Continuity Correction)				

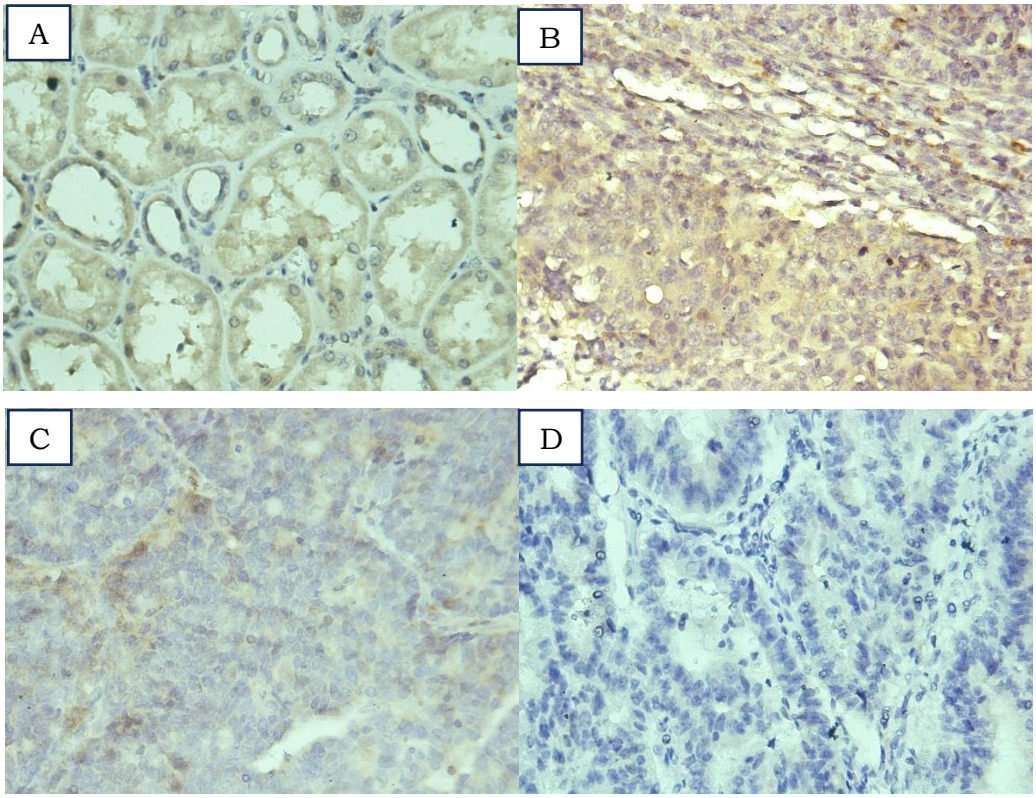

Figure 1. VEGF Expression Patterns in EEC. A. Positive VEGF expression in the positive control, showing brown cytoplasmic staining in tumor cells. B. Strong intensity VEGF expression (Grade 3 EEC). C. Moderate intensity VEGF expression (Grade 2 EEC). D. Weak intensity VEGF expression (Grade 1 EEC).

4. Discussion

This study investigated the expression of vascular endothelial growth factor (VEGF) in endometrioid endometrial carcinoma (EEC) and its association with two critical histopathological prognostic factors: tumor grade and lymphovascular invasion (LVI). The findings revealed that high VEGF expression was significantly correlated with higher histopathological grades of EEC but did not show a statistically significant association with LVI in the studied cohort from Dr. M. Djamil General Hospital Padang. The mean age of EEC patients in this study was 54.36 years, with the highest incidence observed in the 51-60 year age group (41.7%). This is largely consistent with established epidemiological data indicating that EEC predominantly affects perimenopausal and postmenopausal women, often peaking in the fifth to sixth decades. The development of EEC, particularly Type I, which includes most EECs, is frequently linked to prolonged estrogen exposure unopposed by progesterone, leading to endometrial hyperplasia, a precursor lesion. While the average age reported in some studies can be slightly older (around 60 years), our findings align with other studies in Asian populations or specific Indonesian cohorts, which sometimes report a slightly younger age at diagnosis. Factors such as reproductive history, lifestyle, and genetic predispositions might contribute to these regional variations. The youngest patient in our cohort was 28 years old, highlighting that EEC can, albeit less commonly, occur in younger women, often associated with risk factors like polycystic ovary syndrome (PCOS) or hereditary cancer syndromes.[11,12]

In terms of histopathological grade, our study found a predominance of Grade 3 tumors (38.9%), followed by Grade 2 (33.3%) and Grade 1 (27.8%). This distribution, with a higher proportion of high-grade tumors, contrasts with some literature suggesting that lower-grade (Grade 1 and 2) EECs are generally more common. However, our finding is similar to some studies, such as Davidson et al., who also reported a higher percentage of Grade 3 tumors. The higher representation of Grade 3 tumors in this study could be attributed to the fact that Dr. M. Djamil General Hospital Padang is a tertiary referral hospital, which often manages more complex and advanced cases, potentially leading to a selection bias towards higher-grade malignancies.[13,14]

Lymphovascular invasion was detected in 47.2% of the cases, with focal LVI (38.9%) being more frequent than substantial LVI (8.3%). The overall incidence of LVI in EEC can vary widely in literature (reported from 3.2% to 35% in stage I EEC), and our finding of 47.2% (across all stages implicit in a hospital-based sample) is within a plausible range, though perhaps on the higher side, again possibly reflecting the referral nature of the center. LVI is a well-established adverse prognostic factor, linked to lymph node metastasis and recurrence. The thesis noted that LVI, both focal and substantial, was commonly found in Grade 3 tumors, aligning with reports that LVI is more frequent in high-grade disease. The accurate assessment and reporting of LVI, including its extent, are crucial, especially with the new FIGO 2023 staging guidelines. High VEGF expression was found in a significant proportion of cases (58.3%). This is comparable to other studies, which reported high VEGF in over half their cases (9 out of 16), and another study which found VEGF expression in 88% of EEC cases (though the threshold for "high" might differ). VEGF-A is a primary mediator of tumor angiogenesis. Its expression is known to increase from normal endometrium through hyperplasia to carcinoma, suggesting a role throughout endometrial tumorigenesis. VEGF signaling through its receptors, predominantly VEGFR-2, activates multiple downstream pathways promoting endothelial cell proliferation, migration, survival, and vascular permeability, all essential for tumor growth and dissemination. Our observation of frequent high VEGF expression underscores its potential importance in the biology of EEC within this patient population. This study also noted VEGF expression in stromal fibroblasts around the tumor, possibly cancer-associated fibroblasts (CAFs) induced by tumor-secreted cytokines or hypoxia, contributing to the

angiogenic microenvironment.[15,16]

A key finding of this study was the statistically significant positive association between VEGF expression levels and histopathological grade (p=0.000). High VEGF expression was predominantly found in Grade 3 tumors (66.7% of high-VEGF cases were Grade 3), while low VEGF expression was characteristic of Grade 1 tumors (60.0% of low-VEGF cases were Grade 1), and no Grade 3 tumors showed low VEGF expression. This strong correlation suggests that as EEC tumors become less differentiated and histologically more aggressive (higher grade), they tend to exhibit higher levels of VEGF. This implies an increased angiogenic potential in high-grade tumors, which could contribute to their more aggressive biological behavior. This result is consistent with several previous studies. Other studies also reported a significant association between higher VEGF immunoexpression and higher tumor grade in EEC ($p < 0.05$), with high VEGF more common in Grade 3 and low VEGF in Grade 1 tumors. Similarly, another study found a significant correlation between VEGF expression and histological differentiation ($p < 0.05$). Other studies further support this significant relationship. These collective findings reinforce the concept that VEGF-driven angiogenesis is closely linked to the dedifferentiation process and biological aggressiveness inherent in higher-grade EECs. The increased vascularity facilitated by VEGF could provide the necessary nutrients and oxygen for rapid tumor cell proliferation and may also facilitate other aggressive tumor behaviors associated with higher grades.[17,18]

However, it is important to note that not all studies have found such a clear correlation. For example, a study did not find a significant association between VEGF expression and tumor grade (p=0.77). Such discrepancies, as mentioned earlier, can be attributed to variations in sample characteristics (heterogeneity in tumor subtypes if non-EEC are included, distribution of grades), sample size, immunohistochemical techniques, antibody specificity, scoring systems, and statistical approaches. The current study benefited from a homogenous sample of only the EEC subtype and relatively recent tissue blocks (2022-2024), potentially ensuring better tissue antigenicity. Furthermore, the use of a semiquantitative scoring method incorporating both intensity and percentage of stained cells likely provided a more objective assessment of VEGF expression than intensity alone.[19,20]

The mechanisms linking VEGF to higher tumor grade are multifaceted. VEGF, by promoting angiogenesis, supports the increased metabolic demands of rapidly proliferating, poorly differentiated tumor cells. Furthermore, VEGF signaling via VEGFR-2 can directly stimulate tumor cell proliferation and inhibit apoptosis through pathways like MAPK and PI3K/Akt, contributing to tumor progression and aggressiveness. Additionally, co-receptors like Neuropilin-1 (NRP-1), also expressed on tumor and endothelial cells, can modulate VEGF signaling and have been linked to tumor aggressiveness and higher grades in some cancers. Another study showed higher NRP-1 expression in Grade 3 endometrial cancers. While the direct role of NRP-1 in EEC aggressiveness via specific pathways like Ras/ERK or PI3K/Akt is not fully elucidated for EEC, its function as a multifunctional co-receptor enhancing pro-tumorigenic signaling is recognized in other cancers like gastric cancer. The strong correlation observed in our study reinforces the potential of VEGF as an indicator of biological aggressiveness in EEC and supports its further exploration as a prognostic marker for tumor differentiation.

In contrast to the findings with histopathological grade, this study did not find a statistically significant association between VEGF expression and lymphovascular invasion (p=0.080, based on continuity correction). Although a higher percentage of LVI-positive cases (76.5%) had high VEGF expression compared to 23.5% with low VEGF, this difference did not reach statistical significance at the conventional alpha level of 0.05. The Pearson Chi-Square p-value was 0.037, which would be significant; however, the thesis text and conclusion relied on the

corrected p-value. This highlights a point of potential ambiguity in interpretation depending on the statistical test emphasized. Our finding of no significant association (based on p=0.080) aligns with some previous research.Other study, using a similar methodology, also reported that high VEGF expression was significantly linked to tumor grade but not to vascular invasion in EEC. A study (involving 11 studies and 1,251 endometrial cancer patients, though not exclusively EEC or with uniform LVI assessment) found that high VEGF expression correlated with advanced stage, poor differentiation, and lymph node metastasis, but did not show a significant association with LVI. These studies suggest that while VEGF is crucial for overall tumor progression and creating a vascular network that *could* facilitate LVI, its expression level alone might not be the sole or most direct determinant of the actual event of vascular invasion by tumor cells. LVI is a complex process involving tumor cell detachment, degradation of the basement membrane, endothelial transmigration, and survival in the circulation, which likely depends on a concert of molecules beyond just VEGF, including adhesion molecules (E-cadherin downregulation), matrix metalloproteinases (MMPs like MMP-2, MMP-9), and urokinase-type plasminogen activator (uPA). However, other studies have reported conflicting results. Another study suggested a significant association between VEGF expression and LVI, among other clinicopathological features in endometrial cancer. A study demonstrated that increased lymphatic vessel density (LVD), a process driven by lymphangiogenic factors including VEGF-C and VEGF-D (and potentially VEGF-A via VEGFR-2/3 crosstalk or indirect effects), correlated with LVI and lymph node metastasis in EEC, implying an indirect role for the VEGF family in facilitating lymphatic spread. A study found that VEGF-A and VEGFR-2 expression increased with tumor grade and suggested this contributes to LVI.

Moreover, the high prevalence of VEGF expression in EEC, particularly in high-grade tumors, highlights its potential as a therapeutic target. Anti-angiogenic therapies, such as bevacizumab (a monoclonal antibody targeting VEGF-A), have been investigated in advanced or recurrent endometrial cancer, with some studies suggesting benefits in progression-free survival when combined with chemotherapy, although its role in routine clinical practice is still evolving and often reserved for specific scenarios. Identifying which patients are most likely to respond to anti-VEGF therapy is crucial, and tumor VEGF expression levels could be a part of this patient selection process. Routine evaluation of VEGF, as suggested by the thesis, might therefore be considered, particularly in high-grade EEC, to guide therapeutic considerations.

Future research should aim to address the limitations of the current study. Larger, prospective studies incorporating standardized methodologies for LVI assessment (including IHC markers), comprehensive molecular subtyping of EEC, and long-term clinical follow-up are needed. Investigating the expression of different VEGF isoforms (VEGF-A, -C, -D) and their respective receptors (VEGFR-1, -2, -3), as well as other angiogenic and lymphangiogenic factors, would provide a more complete picture of the vascular biology of EEC. Exploring the interplay between VEGF signaling, the tumor microenvironment (including immune cells and CAFs), and molecular subtypes of EEC will be essential for advancing our understanding and developing more effective, personalized anti-angiogenic strategies. The correlation of VEGF expression not just with pathological features but directly with patient survival rates, progression-free survival, and response to specific therapies will be critical in establishing its definitive prognostic and predictive value in EEC management.

5. Conclusion

Endometrioid endometrial carcinoma in this study population was most frequently diagnosed in women aged 51-60 years, with a mean age of 54.36 years. The cases were predominantly high-grade (Grade 3), and a notable proportion exhibited LVI, mostly focal in nature. High VEGF expression was observed in the majority of tumor samples. The increased VEGF-

mediated angiogenesis is linked to poorer tumor differentiation and potentially more aggressive biological behavior in EEC. These findings contribute to the understanding of the role of VEGF in EEC, particularly highlighting its association with tumor dedifferentiation.

6. References

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