Hypomethylation of the Promoter Region of the Vascular Endothelial Growth Factor (VEGF) Gene and It's Low Expression in Placenta as a Risk Factor for Preeclampsia

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Abstract

Objective: This study aimed to determine the association between hypomethylation of VEGF gene promoter region and low VEGF expression in the placenta as a potential risk factor for preeclampsia.
Methods: An observational case-control study was conducted involving pregnant women with preeclampsia and normotensive controls. Placental tissue samples were collected and analysed for DNA methylation status of the VEGF gene promoter using quantitative real-time PCR. VEGF expression levels in placental tissue were measured using ELISA.
Results: Significant hypomethylation of the VEGF promoter region was observed in placenta samples from preeclamptic women compared to controls. This finding correlated with decreased VEGF expression. These results indicate that reduced VEGF expression due to promoter hypomethylation may impair placental vascularization, contributing to preeclampsia.
Conclusions: Hypomethylation of the VEGF promoter and subsequent reduction in placental VEGF expression may serve as early biomarkers to predict preeclampsia. These results highlight the importance of epigenetic regulation in preeclampsia pathogenesis and suggest potential targets for early diagnosis and intervention.

Keywords: Preeclampsia; VEGF; Hypomethylation; Placenta; Epigenetics

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Hipometilasi Regio Promoter Gen Vascular Endothelial Growth Factor (VEGF) dan Ekspresi Vascular Endothelial Growth Factor (VEGF) yang Rendah pada Plasenta sebagai Faktor Risiko Preeklampsia

Abstrak
Tujuan: Penelitian ini bertujuan untuk mengetahui hubungan antara hipometilasi pada daerah promoter gen *VEGF* dan rendahnya ekspresi *VEGF* di plasenta sebagai faktor risiko potensial terjadinya preeklampsia.
Metode: Penelitian observasional dengan desain kasus-kontrol ini melibatkan ibu hamil dengan preeklampsia dan kontrol normotensif. Sampel jaringan plasenta dikumpulkan dan dianalisis untuk menentukan status metilasi DNA pada daerah promoter gen *VEGF* menggunakan *quantitative real-time PCR*. Tingkat ekspresi *VEGF* dalam jaringan plasenta diukur menggunakan metode ELISA.
Hasil: Didapatkan hipometilasi yang signifikan pada daerah promoter gen *VEGF* pada jaringan plasenta ibu dengan preeklampsia dibandingkan kelompok kontrol. Temuan ini berkorelasi dengan penurunan ekspresi *VEGF*. Hasil tersebut menunjukkan bahwa penurunan ekspresi *VEGF* akibat hipometilasi promoter dapat mengganggu vaskularisasi plasenta, sehingga berperan dalam patogenesis preeklampsia.
Kesimpulan: Hipometilasi pada daerah promoter gen *VEGF* dan penurunan ekspresi *VEGF* di plasenta dapat berfungsi sebagai biomarker awal untuk memprediksi terjadinya preeklampsia. Hasil ini menyoroti pentingnya regulasi epigenetik dalam patogenesis preeklampsia serta membuka peluang untuk diagnosis dini dan intervensi yang lebih tepat.

Kata kunci: Preeklampsia; *VEGF,* Hipometilasi, Plasenta; Epigenetik

Introduction

Preeclampsia is a pregnancy-related disorder that manifests with new-onset hypertension after 20 weeks of gestation and is one of the major contributor of obstetric complications namely maternal organ dysfunction and fetal growth restriction. Despite significant advancements in prenatal care, preeclampsia remains one of the leading causes of maternal and neonatal morbidity and mortality. World Health Organization estimated that preeclampsia accounts for 18% of maternal deaths globally, with the incidence particularly higher in developing countries, including Indonesia.[1] In Bali, the prevalence of preeclampsia is 4.91%, with a case fatality rate (CFR) of 24%.[2]

The pathogenesis of preeclampsia is not fully understood, but evidence suggests an imbalance in angiogenesis, particularly involving Vascular Endothelial Growth Factor (VEGF), a potent angiogenic protein.

Normally, VEGF-A will bind its receptor (VEGF-Receptor) to induce angiogenesis, causing transient calcium influx and increasing vascular permeability.[3] When trophoblast invasion mechanism is disturbed, sFLT-1 (soluble Fms-like tyrosine kinase-1), a potent anti-angiogenic factor binds to and inhibits vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which are crucial for endothelial cell function and vascular remodeling during pregnancy.[4]

This study focuses on the role of epigenetic modifications, specifically the hypomethylation of the VEGF promoter region, which leads to its low expression, which subsequently contribute to the pathogenesis of preeclampsia.[3] Early detection and intervention in preeclampsia is critical, as it may significantly improve maternal and fetal outcomes. Previous studies have suggested that DNA methylation, especially hypomethylation of the VEGF gene promoter, plays a crucial role in the regulation of VEGF expression.[4] Understanding these epigenetic modifications may lead to the development of novel biomarkers for the early detection of preeclampsia and targeted therapeutic strategies.

This study aims to investigate the hypomethylation of the VEGF gene promoter as a risk factor for preeclampsia and explore the relationship between low VEGF expression and the development of the disease. The findings could have important implications for the prevention and management of preeclampsia, particularly in regions with high prevalence, such as Indonesia.[5]

Methods

This is a case-control study with aim to investigate the relationship between hypomethylation of the VEGF gene promoter region and low VEGF expression in women with preeclampsia. The study was conducted at Prof. Dr. I.G.N.G. Ngoerah General Hospital in Denpasar, Bali, Indonesia, from January to December 2024. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Udayana University, ensuring that all patient data were handled confidentially and with informed consent (Ethical Clearance number: 045/UN14.2.2.VII.14/LT/2024).

Participants of the study are pregnant women diagnosed with preeclampsia, with normotensive pregnant women as controls. Inclusion criteria are as follows: (i) Age 18-40 years; (ii) diagnosed with preeclampsia based on ACOG criteria, defined by systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation, with proteinuria or other signs of organ dysfunction; (iii) singleton pregnancy; (iv) provided informed consent. Exclusion criteria are as follows: (i) multiple gestation; (ii) history of maternal diseases such as diabetes mellitus, systemic infections, autoimmune disorders, or other hypertensive disorders not related to pregnancy; (iii) history of medication usage that could interfere with gene expression, such as corticosteroids or immunosuppressants.

The sample size was calculated using a **confidence level of 95%** and a **power of 80%** to detect a statistically significant difference in the **methylation status** and **expression levels** of VEGF. Based on previous studies and estimated effect sizes, the minimum required sample size for each group was 30 participants. A total of 60 women (30 with preeclampsia and 30 normotensive controls) were included in the study.

The tools used for the study are as follows:

1. **DNA Extraction Kit**: QIAamp DNA Mini Kit (Qiagen, Germany) for extracting genomic DNA from placental tissue.
2. **RNA Extraction Kit**: TRIzol reagent (Invitrogen, USA) for extracting total RNA from placental tissue samples.
3. **Bisulfite Modification Kit**: EZ DNA Methylation-Gold Kit (Zymo Research, USA) for DNA methylation analysis.
4. **Primers for MSP**: Custom primers designed for **methylation-specific PCR** (MSP) targeting the VEGF **gene promoter region**.
5. **SYBR Green Master Mix**: For **quantitative real-time PCR (qRT-PCR)** to quantify **VEGF gene expression** (Applied Biosystems, USA).
6. **Surgical Instrumentation**: For obtaining placental tissue samples during delivery.
7. **Tumor Marker Kits**: **AFP**, **hCG** levels were analyzed using **ELISA** (Enzyme-Linked Immunosorbent Assay) kits (BioSource International, USA).

Placental tissue samples were collected from participants during cesarean sections or vaginal deliveries, specifically from the maternal side of the placenta within 30 minutes after delivery to minimize degradation. The tissues were immediately placed in RNAlater (Ambion, USA) for RNA stabilization and stored at -80°C until further analysis. Genomic DNA was extracted using the QIAamp DNA Mini Kit following the manufacturer's protocol, and DNA quality was assessed with a NanoDrop spectrophotometer (Thermo Scientific, USA). One microgram of DNA was subjected to bisulfite conversion using the EZ DNA Methylation-Gold Kit (Zymo Research), which converts unmethylated cytosines to uracil while leaving methylated cytosines unchanged. Methylation-specific PCR (MSP) was performed using primers designed to specifically amplify methylated or unmethylated regions of the sFLT-1 promoter. PCR reactions were carried out in a 50 µL volume containing Taq polymerase and 5 µL of bisulfite-treated DNA, using a thermal cycler (Applied Biosystems, USA) with the following conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. PCR products were separated on a 2% agarose gel and visualized under UV light to determine methylation status. VEGF expression analysed with enzyme-linked immunosorbent assay (ELISA) (Catalog number HUFI00281; Assay Genie: enzyme-linked immunosorbent assays).

Data analysis was conducted using SPSS version 26.0 (IBM Corp, USA). Demographic characteristics of the participants were presented using descriptive statistics, including mean and standard deviation. Comparisons of methylation levels and gene expression between preeclampsia and normotensive groups were performed using independent t-tests or Mann-Whitney U tests as appropriate. Logistic regression analysis is used to determine the association between VEGF promoter hypomethylation and the risk of developing preeclampsia. Statistical significance was defined as p-value less than 0.05.

Result

This study included 60 pregnant women, with 30 preeclamptic women and 30 normotensive controls. Age, parity, and body mass index (BMI) were compared across groups (Table 1). Normality tests showed no significant differences in age, parity, or BMI ($p > 0.05$). The mean age was 29.57 ± 7.7 years in the preeclampsia group and 27.40 ± 6.43 years in the control group ($p = 0.214$). However, pregnant women over 35 years had a significantly higher risk of preeclampsia (OR 7.00, 95% CI 1.38-35.47, $p = 0.021$). The mean number of children was 1.73 ± 1.28 in the preeclampsia group and 1.07 ± 0.91 in the control group ($p = 0.089$). Parity ≥1 did not show a significant association with preeclampsia (OR 0.36, 95% CI 0.09-1.33, $p = 0.117$). The mean BMI in the preeclampsia group was 24.66 ± 1.93 kg/m^2, significantly higher than the control group (22.05 ± 1.58 kg/m^2, $p < 0.001$). A BMI ≥25 kg/m^2 was associated with a significantly higher risk of preeclampsia (OR 9.33, 95% CI 2.84-30.60, $p < 0.001$).

In this study, placental tissue samples from pregnant women with preeclampsia showed 1 (3.3%) unmethylated and 29 (96.7%) partially methylated VEGF gene promoters. In contrast, all 30 placental samples from non-preeclamptic women had partial methylation. DNA methylation levels were quantified using ImageJ software, which analyzed the electrophoresis band intensity. The average DNA methylation percentage of the VEGF gene in preeclamptic placental samples was 30.36 ± 11.45%, while in non-preeclamptic placental samples it was 48.10 ± 13.48%. Independent T-test analysis revealed a significant difference in methylation levels between the two groups (p

Table 1 Relationship between Subject Characteristics and the Incidence of Preeclampsia.

Characteristics	Preeclampsia (n= 30)	Non-Preeclampsia (n=30)	OR	CI 95%	p
Age (years)					
Mean ± SD	29.57 ± 7.7	27.40 ± 65.43			0.214 [a]
Age (years), n(%)					
≤20	5 (16.7)	2 (6,7)	7.00	1.38-35.47	0.021 [b] *
21-35	17 (56.7)	26 (86.7)			
>35	8 (26.7)	2 (6,7)			
Parity					
Mean ± SD	1.73 ± 1.28	1.07 ± 0.91			0.089 [a]
Parity					
Nulliparous	4 (13.3)	9 (30.0)	0.36	0.09-1.33	0.117 [b]
Parity ≥1	26 (86.7)	21 (70.0)			
BMI (kg/m2)					
Mean ± SD	24.66 ± 1.93	22.05 ±1.58			<0.001 [a] *
BMI (kg/m ^{2})					
<18.5	0 (0)	1 (3,3)	9.33	2.84-30.60	<0.001 [b] *
18.5-22.9	9 (30.0)	23 (76.7)			
23,24,9	7 (23.3)	5 (16.7)			
≥25	14 (46.7)	1 (3,3)			

[a] Independent T test, [b] Chi square test , *

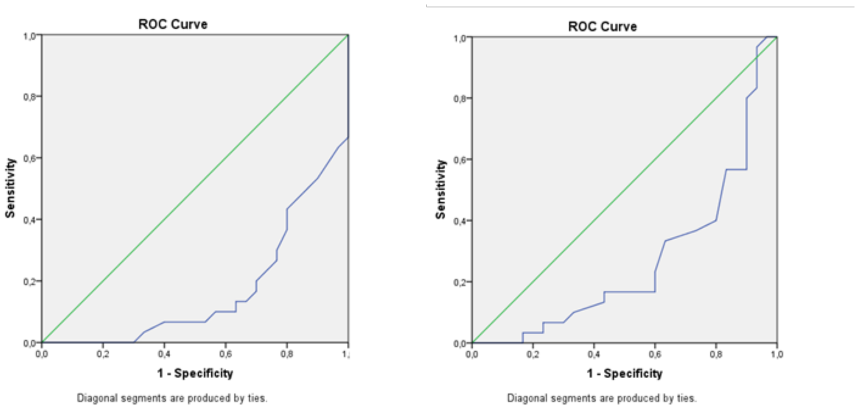

Figure 1 ROC curve analysis of the Percentage of DNA Methylation of the VEGF Gene Promoter for Predicting the Incidence of Preeclampsia (a) & ROC curve analysis of VEGF expression for predicting preeclampsia incidence (b)

Table 2 Relationship between VEGF Gene Promoter DNA Hypomethylation and the Incidence of Preeclampsia

VEGF Gene Promoter Methylation	Preeclampsia (n= 30)	Non preeclampsia (n=30)	OR	CI 95%	p
Hypomethylation (<37%)	22 (73.3%)	7 (23.3%)	9.0	2.80 – 29.13	<0.001
Normal methylation (≥37%)	8 (26.7%)	23 (76.7%)			

< 0.001), indicating hypomethylation of the VEGF gene in preeclamptic pregnancies.

To further assess the role of VEGF gene promoter hypomethylation as a risk factor, ROC curve analysis identified an optimal cutoff of 37% for DNA methylation. Pregnant women with preeclampsia were more likely to have VEGF promoter hypomethylation (<37%) compared to those without preeclampsia **(Figure 1a)**. Chi-square analysis (**Table 2**) confirmed that hypomethylation of the VEGF gene promoter significantly increased the risk of preeclampsia, with an Odds Ratio (OR) of 9.0 (95% CI 2.80-29.13, p < 0.001).

ROC curve analysis identified an optimal VEGF expression cutoff of 4.62 pg/ml, with an AUC of 0.734, sensitivity of 73%, and specificity of 63% **(Figure 1b)**. VEGF expression was categorized as low (≤4.62 pg/ml) or high (>4.62 pg/ml). The data showed that 63.3% of preeclamptic women had low VEGF expression, while 73.3% of non-preeclamptic women had high VEGF expression. Low VEGF expression significantly increased the risk of preeclampsia by 4.75 times (OR 4.75; 95% CI 1.58 – 14.2; p = 0.004), group with VEGF gene promoter hypomethylation tends to have low VEGF levels (66.7%), while the group with normal methylation tends to have high VEGF levels (66.7%). The analysis showed that VEGF gene promoter hypomethylation significantly increased the risk of low VEGF levels by 4.0 times (OR 4.0; 95% CI 1.36 – 11.76; p = 0.010).as shown in **Table 3**.

The normality test for VEGF data revealed a non-normal distribution (p < 0.001), so the Mann-Whitney test was used to compare VEGF expression between pregnant

Table 3 Relationship between VEGF Serum Level and VEGF Promoter Methylation in Preeclampsia

Variable	Preeclampsia (n=30)	Non-Preeclampsia(n=30)	OR	95% CI	p-value
VEGF Serum Level					
Low (≤4.62 pg/mL)	19 (63.3%)	8 (26.7%)	4.75	1.58 – 14.2	0.004
High (>4.62 pg/mL)	11 (36.7%)	22 (73.3%)			
VEGF Gene Promoter Methylation					
Hypomethylation (<37%)	18 (66.7%)	11 (33.3%)	4.00	1.36 – 11.76	0.010
Normal Methylation (≥37%)	9 (33.3%)	22 (66.7%)			

Table 4 Multivariate Analysis for Preeclampsia Risk Factors.

Risk Factors	*Adjusted* OR (AOR)	CI 95%	p
Maternal age >35 years	3.47	0.49-24.62	0.212
Nulliparous	0.45	0.05-3.88	0.472
Obesity (BMI >25 kg/m ^{2})	11.56	2.02-66.06	0.006*
Low VEGF expression (≤4.62 pg/ml)	7.21	1.40-37.0	0.018*
VEGF DNA hypomethylation (<37%)	10.77	2.23-51.94	0.005*

* Significant results

women with preeclampsia and those without. The results showed that pregnant women with preeclampsia had significantly lower VEGF levels (5.49 ± 6.77 pg/ml) compared to non-preeclamptic women (27.05 ± 57.05 pg/ml, p = 0.002).

A multivariate logistic regression analysis was conducted to assess the impact of low VEGF expression and VEGF gene promoter hypomethylation on preeclampsia, adjusting for confounding factors such as age, parity, and BMI. The results are presented in Table 4. The analysis showed that both low VEGF expression and VEGF gene promoter DNA hypomethylation remained significant risk factors for preeclampsia after controlling for age, parity, and BMI. Specifically:

- Low VEGF expression (≤4.62 pg/ml) increased the risk of preeclampsia by 7.21 times (aOR 7.21; 95% CI 1.40-37.0; p = 0.018).
- VEGF gene promoter DNA hypomethylation increased the risk by 10.77 times (aOR 10.77; 95% CI 2.23-51.94; p = 0.005).

Discussion

The present study aimed to explore the role of hypomethylation of the VEGF gene promoter region and as a potential risk factor for preeclampsia and to evaluate the relationship between low VEGF expression and the pathogenesis of this disorder. We found that hypomethylation of the VEGF gene promoter and low VEGF expression were significantly associated with the occurrence of preeclampsia. These findings suggest that epigenetic modifications, specifically DNA methylation, may play a critical role in the development of preeclampsia, and they underscore the potential of VEGF as a biomarker for early detection.

Vascular Endothelial Growth Factor

(VEGF) is a potent angiogenic factor which are crucial for mitogenic, endothelial anti-apoptosis, increasing vascular permeability, and cell migration. VEGF-A is the most potent angiogenesis will bind it's receptor (VEGF-Receptor) for angiogenesis process, causing transient calcium influx and increasing vascular permeability. A study by Sara et al. (2024) explains that hypoxia and inflammation in endothelial will induce angiogenesis through endothelial proliferation, increasing permeability with aim to fix endothelial vascularization.[6]

When the trophoblast invasion mechanism is disturbed, the sFLT-1 (soluble Fms-like tyrosine kinase-1),a potent anti-angiogenic factor binds to and inhibits vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which are crucial for endothelial cell function and vascular remodeling during pregnancy.[7]

Our study revealed that hypomethylation of the VEGF promoter led to low expression of this angiogenic factor, which is consistent with previous reports suggesting that epigenetic alterations contribute to the pathophysiology of preeclampsia The significant association between hypomethylation and low VEGF expression aligns with previous studies, such as those by Sundrani et al. (2013), who found that low VEGF levels were a hallmark of preeclampsia, contributing to the imbalance of angiogenic factors.[6] Our findings extend these observations by providing evidence that DNA methylation may be one of the regulatory mechanisms driving low expression of VEGF in preeclamptic placental tissue. Additionally, the statistical significance observed in our results supports the hypothesis that hypomethylation of the VEGF promoter could serve as an early molecular marker for preeclampsia. Previous studies have shown that VEGF levels correlate with the severity of preeclampsia, and monitoring these levels may help in identifying at-risk pregnancies before the onset of clinical symptoms. This is in line with the work of Dave et al. (2021), who found that VEGF expression is tightly regulated by epigenetic modifications in placental tissue.[7,8]

Our findings are consistent with earlier studies that identified epigenetic regulation of VEGF as a key player in preeclampsia. For instance, Sundrani et al. (2013) showed that altered DNA methylation patterns in genes related to angiogenesis, including VEGF, were present in early-onset preeclampsia.[8] Similarly, Hernandez and Gedeon (2019) highlighted the role of DNA methylation in regulating genes involved in vascular health during pregnancy, further supporting our findings.[9,10] However, our study also differs from others in several key areas. While many previous studies have focused on the circulating levels of VEGF as a diagnostic marker, our study provides novel insights into the epigenetic regulation of this gene, showing that promoter hypomethylation contributes directly to its overexpression in preeclamptic placental tissue.[11,12] This adds a new layer of understanding to the molecular mechanisms behind the disease.[12,13]

Additionally, while other studies have shown the predictive value of VEGF levels in preeclampsia, our research suggests that epigenetic markers could complement these traditional biomarkers and provide more reliable early warning signs of the disease[14]. This aligns with the work of Thompson et al. (2021), who proposed the use of genetic and epigenetic markers to predict preeclampsia before clinical symptoms manifest.[13-15]

The results of this study open up several important avenues for future research. One promising direction is the exploration of non-invasive tests to detect hypomethylation of VEGF in maternal blood, which could serve as a biomarker for early detection of preeclampsia. Recent advances in liquid biopsy technologies and the detection of circulating DNA or epigenetic markers in maternal plasma may facilitate non-invasive prenatal screening for preeclampsia. Another potential area for future investigation is the development of epigenetic therapies aimed at modulating VEGF expression in women at risk of preeclampsia. By targeting specific epigenetic modifications, such as DNA methylation inhibitors, it may be possible to prevent or treat preeclampsia more effectively, improving maternal and fetal outcomes.

Moreover, longitudinal studies are needed to explore the dynamic changes in VEGF methylation during pregnancy and their correlation with the development of preeclampsia. This would provide valuable insights into the temporal relationship between epigenetic modifications and the onset of preeclampsia, helping to refine diagnostic criteria and improve patient management.

Conclusion

In conclusion, this study provides compelling evidence that hypomethylation of the VEGF gene promoter is a significant risk factor for VEGF low expression and plays a critical role in the pathogenesis of preeclampsia. These findings suggest that epigenetic regulation of VEGF could serve as an early biomarker for preeclampsia, providing opportunities for improved diagnosis and treatment. Future research should focus on developing non-invasive diagnostic tests and exploring epigenetic therapies to prevent or mitigate the effects of preeclampsia, ultimately improving maternal and fetal health outcomes.

Conflict of Interest

No Conflict of Interest.

Advice and Thanks

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AI Disclosure

The author declares that this study did not use any generative AI tools in the process of manuscript writing.

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