RESEARCH ARTICLE

miR-200a as Potential Early-onset Biomarker, while High Nitric Oxide as Potential Late-onset Biomarker in Preeclampsia

Hudila Rifa Karmia[1,*], Yusrawati[1], Jamsari[2], Hardisman[3]

[1]Division of Fetomaternal, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Andalas, Limau Manis, Pauh, Padang 25163, Indonesia
[2]Faculty of Agricultural, Universitas Andalas, Limau Manis, Pauh, Padang 25163, Indonesia
[3]Department of Public Health, Faculty of Medicine, Universitas Andalas, Limau Manis, Pauh, Padang 25163, Indonesia

*Corresponding author. Email: hudilarifakarmia@med.unand.ac.id

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Abstract

BACKGROUND: miR-200a is known to alter trophoblast invasion and spiral artery remodeling, leading to defective placentation that causes placental hypoxia, which is the main pathomechanism in early-onset preeclampsia (EOPE). Hypoxic placentas cause systemic endothelial dysfunction that is characterized by low production of endothelial vasodilator, mainly nitric oxide (NO). On the other hand, defective placentation does not cause late-onset preeclampsia (LOPE), making the role of miR-200a expression and NO level as predictors in LOPE questionable. Therefore, this study was conducted to compare miR-200a expressions and NO levels in EOPE and LOPE to clarify their role in pathomechanism of both types of preeclampsia.

METHODS: A cross-sectional comparative study was conducted in 62 preeclamptic patients (31 EOPEs and 31 LOPEs). Subjects were classified into EOPE or LOPE groups based on whether the diagnosis of preeclampsia was made at <34 or ≥34 weeks of pregnancy. miR-200a expression was analyzed using real-time polymerase chain reaction technique, and NO level was analyzed using colorimetric assay method.

RESULTS: EOPE and LOPE subjects were equivalent in terms of age and parity (p=0.709 and p=0.066), but significantly difference in gestational age (p=0.000). miR-200a were expressed in 74.2% of EOPE and 41.9% of LOPE subjects (p=0.010). Median NO levels were lower in EOPE compared to LOPE subjects (23.75 *vs.* 106.00 µmol/L) (p=0.027), and lower in subjects with detected miR-200a compared to subjects with undetected miR-200a (62.75 *vs.* 132.25 µmol/L) (p=0.032).

CONCLUSION: miR-200a was more expressed in EOPE compared to LOPE subjects suggesting that it might be a significant in predicting EOPE. While NO level was significantly lower in EOPE whilst higher in LOPE subjects, hence might be potential as a marker to differentiate EOPE and LOPE.

KEYWORDS: miR-200a expression, NO level, early-onset preeclampsia, late-onset preeclampsia

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Introduction

Preeclampsia is a new-onset hypertension and new-onset end-organ damage, complicating pregnancy of >20 weeks gestation. While the pathophysiology of this complex process involves multiple organ systems (1), many studies only focus on the diagnosis and pharmacological treatment of preeclampsia (2), thus it leads the disease to be the main cause of pregnancy-related complications (3). The exact pathomechanism and pathophysiology of the disease have not been fully understood yet (4), but it is supposed that the initial process of pathogenesis starts in the beginning of placentation, which involves trophoblast differentiation,

division, migration and invasion (1). Impaired trophoblast invasion leading to incomplete spiral artery remodeling is responsible for the initial process of placentation defect.(1) Hypoxic placenta indirectly caused by incomplete spiral artery remodeling releases placental factors into maternal blood stream.(5) Therefore, to fully understand the disease, study focusing on early events of placental development should be performed.(6)

In recent years, it was found that several micro-RNAs (miRNAs) are highly expressed in most placental originated diseases, but the exact mechanism has not been understood yet.(7) Of 55 exosomal miRNAs studied, miR-1283 has the highest expression in preeclampsia (8), but there was no specific data about miR-200a expression. miR-210 was more expressed in EOPE subjects compare to LOPE subjects.(9) miR-200a was more expressed in preeclamptic subjects compare to normal pregnancy subjects, whilst high miR-200a serum expressions was reported as a good predictor for preeclampsia with the sensitivity and specificity of 87.3% and 96.0% respectively, and was associated with increased adverse pregnancy outcome (10), however the comparison of miR-200a expression between EOPE and LOPE remains unclear. High miR-200a expression leads to impaired trophoblast invasion (11,12), a process that underlies the failure of spiral artery remodeling, which will then lead to hypoxic placenta and endothelial dysfunction. It means that miR-200a might have a role in early-onset preeclampsia (EOPE) is as initiator of impaired trophoblast proliferation.

Endothelial dysfunction is characterized by low production of endothelium-derived relaxing factors (EDRF), mainly nitric oxide (NO), leading to imbalance between EDRF and endothelium-derived contractile factors (EDCF) productions and bioavailabilities. Decreased nitric oxide (NO) production lead to elevated vascular tone and hypertension.(13) Thus, low NO level may be used as an indicator that the pathogenesis of preeclampsia has already been in the endothelial dysfunction condition (the beginning of stage-2 preeclampsia pathogenesis).

While defective placentation is related to EOPE, on the other hand, defective placentation does not cause late-onset preeclampsia (LOPE). High miR-200a has an anti-inflammatory effect on the vascular system (11), whilst an increasing systemic (vascular) inflammatory response in pregnancy is a main etiopathogenesis of LOPE (14). Due to the anti-inflammatory effect of miR-200a, it can be assumed that miR-200a might suppresses/prevents the occurrence of LOPE. However, the detailed mechanisms regarding the different between the two types of preeclampsia still need to be studied.

Given the difference role of miR-200a and NO in the pathomechanism of EOPE and LOPE, the role of high miR-200a expression as predictor, and low NO level as a marker of endothelial dysfunction in preeclampsia pathogenesis and as a cause of hypertension in LOPE remains questionable. Therefore, it is necessary to study the expression of miR-200a and the level NO in preeclamptic patients, specifically EOPE and LOPE patients. This study was conducted to compare and to analyze miRNA-200a expressions and NO levels in EOPE and LOPE to clarify the role of miR-200a in the pathomechanism, and the role of NO in the basic mechanism of hypertension.

Methods

Study Design and Subjects Reqruitment

An observational study with a cross-sectional comparative study design, was conducted to compare miR-200a expressions and NO levels between EOPE and LOPE subjects. A total 62 cases of preeclamptic patients at Dr. M. Djamil Hospital Padang City were enrolled as subjects in this study. Subjects were classified as EOPE subjects if the diagnosis of preeclampsia was made at <34 weeks of pregnancy, whilst as LOPE subjects if the diagnosis was made at ≥34 weeks of pregnancy. There were 31 EOPE and 31 LOPE subjects. Subjects of EOPE whose pre-pregnancy diagnosed as suffered from hypertension, diabetes mellitus, metabolic syndromes, or autoimmune diseases were excluded from this study.

Data about subjects' age, gestational age, gravidity, parity, body mass index, blood pressure and vital signs were collected using primary survey, while the laboratory findings including hemoglobin, leucocyte, thrombocyte, liver function test, renal function test, hemostatic function test, and blood glucose were collected as blood samples from the subjects' vena cubiti. The protocol of this study was approved by Health Research Ethics Committee RSUP Dr. M. Djamil Padang (No DP.43/D.XVI.XI/512/2023, dated on September 21, 2023).

miR-200a Expression Examination

Trizol reagent (Invitrogen, Waltham, MA, USA) was used to extract total RNA from the sample, and then by using the Revert Aid First Stand cDNA synthesis kit (miCURY LNA RT Kit-339340; Qiagen, Hilden, Germany), the isolated RNA was reverse transcripted into complementary DNA (cDNA). The expression of miR-200a-3p was determined by quantitative real-time polymerase chain reaction (qRT-

PCR) with miRCURY SYBR master mix (Qiagen), in according with the manufacturer's instructions. $2^{-\Delta\Delta Cq}$ method was used to calculate the relative expression of miR-200a-3p. The primers used were: miR200a-3p forward: 5′-TAACACTGTCTGGTAACGATGT and reverse: 5′-CATCTTACCGGACAGTGCTGGA; U6 forward: 5′- CTCGCTTCGGCAGCACA and reverse: 5′AACGCTTCACGAATTTGCGT.(15) miR-200a was referred as "detected" if it was expressed in the 30-50 cycles of amplification and was referred as "undetected" if it was not expressed within 50 cycles of amplification. Relative Fluorescence Units (RFU) that indicates the amount of fluorescence emitted by sample relative to a reference sample or a standard curve, was used to relatively quantify miR-200a expression. By conversion of RFU value, it was found that miR-200a expression was between 0.00–49.48 ng/mL. The miR-200a amplification graph was shown in Figure 1.

NO Levels Examination

Serum NO levels were measured by colorimetric method, using NO Colorimetric Assay Kit (Catalog No: E-BC-K035-M; Elabscience, Houston, TX, USA). NO level was indirectly measured by nitrate or nitrite, by measuring the OD value at 550 nm. The examination was done in accordance with the procedure given by manufacturer. Standard curve was prepared by diluting 2 mmol/L sodium nitrite standard with double distilled water to a serial concentration. The dilution gradient was as follows: 0, 10, 20, 30, 40. 60, 80, 100 µmol/L. As much as 200-300 µL of sodium nitrite standard solution was taken with different concentrations to 1.5 mL EP tubes, then 200 µL of reagent-1 was added and mixed fully with a vortex mixer. One hundred µL of reagent-2 was added and mixed again before being put in room temperature for 15 min and centrifuged again at 300 g for 10 min. As much as 160 µL of supernatant was taken to the corresponding wells of microplate for chromogenic reaction. Eighty 80 µL of chromogenic reagent was added to each well and oscillated for 2 min and put in room temperature for 15 min. The concentration of NO was calculated according to the formula based on the OD value of the sample.

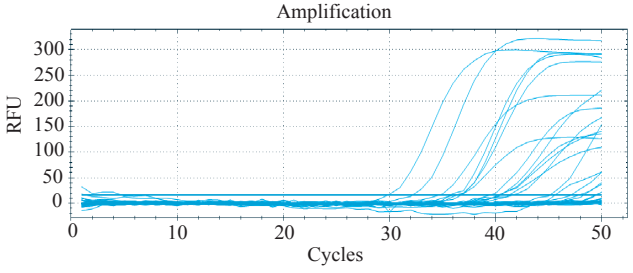

Figure 1. miR-200a amplification. miR-200a gen expressed within 30-50 cycles. Process of amplification was stopped after 50 cycles. RFU: Relative Fluorescence Units.

Results

Characteristics of Subjects

Characteristics of subjects included were participant's age, gestational age, and parity as presented in Table 1. Both subjects in the EOPE and LOPE were similar in the context of age (p=0.709) and parity number (p=0.066), but were significantly differed in the context of gestational age (p=0.000) with LOPE subjects having older gestational compared to EOPE subjects.

miR-200a was More Expressed in EOPE

There was a significant difference of detected miR-200a in EOPE and LOPE subjects (p=0.010; CI=95%; OR=3.98(1.36-11.67)). miR-200a expression was detected in 23 of 31 EOPE (74.2%) and in 13 of 31 LOPE (41.9%) subjects, while the remaining subjects showed undetected miR-200a expression. There was also a significant difference of miR-200a expression median in EOPE and LOPE subjects (p=0.048) (Table 2).

NO Level was Higher in LOPE

There was significant difference of NO level (p=0.027), with median NO level in LOPE subjects was higher compared to the EOPE subjects (106.00 *vs.* 23.75 µmol/L) (Table 2).

NO Level in Subjects with Detected and Undetcted miR-200a Expression

To confirm whether the difference of NO levels was associated to miR-200 expression, further analyzed was performed. NO level in subject with detected miR-200a expression and in subjects with undetected miR-200a expression showed a significant difference with p=0.032 (62.75 *vs*. 132.25 µmol/L, respectively) (Table 3).

Discussion

Table 2 shows that miR-200a were expressed in 74.2% of EOPE samples and in 41.9% of LOPE samples with the median expression of 36.75 ng/mL and 0.00 ng/mL respectively, which was significantly different. miR-200a

Table 1. Characteristics of subjects.

Characteristics	Onset of Preeclampsia		*p*-value
	EOPE (n=31)	LOPE (n=31)	
Age (years), mean±SD	30.26±5.58	30.81±5.93	0.709[a]
Gestational age (weeks), mean±SD	30.42±2.92	37.45±2.30	0.000*[a]
Parity			
Primiparous	14 (45.2%)	13 (41.9%)	0.066[b]
Multiparous	17 (54.8%)	18 (58.1%)	

*Significant if $p<0.05$, [a]Tested with t-test; [b]Tested with Chi-square test.

expression in EOPE subjects is reported to be higher than median miR-200a expression in preeclampsia (*i.e.*, 2.136 ng/mL) (11) and mean miR-200a expression in preeclampsia (*i.e.*, 1.655 ng/mL) (10). It suggests that miR-200a has an important role in the pathogenesis of EOPE. miR-200a family was associated with preeclampsia and was known to be upregulated in the placenta and plasma of preeclamptic patient.(16) miR-200a downregulates insuline-like growth factor-2 (IGF2) protein expression in trophoblast cells until 80% by directly targets Igf2 3'-UTR and represses its expression. miR-200a may also regulates endogenous IGF2 protein level through suppression of Akt pathway, leading to altered cell survival, division, and differentiation. This downregulation of IGF2 protein may lead to inadequate trophoblast invasion, inadequate spiral artery remodeling, and inadequate placentation.(17) Increased expression of miR-200a inhibits the expression of endocrine gland derived-VEGF (EG-VEGF), and suppresses the extracellular signal-regulated kinase (ERK) pathway, so that trophoblast invasion is disrupted.(11) Decreased levels of pro-angiogenic factor (mainly VEGF-A) may be aggravated by genetic variant of VEGF-A C/A gene, since it was associated with preeclampsia.(18) miR-200a inhibitors strongly suppressed miR-200a expression and increased proliferation, migration, and invasion, as well as suppressed trophoblast apoptosis.(12)

The hypoxic placenta releases anti-angiogenic factors (mainly sFlt-1) into the maternal circulation. sFlt-1 level was significantly higher in EOPE compared to LOPE.(19) Increased anti-angiogenic factor levels (7) accompanied by decreased pro-angiogenic factor levels lead to higher anti-angiogenic to pro-angiogenic ratio; and this may cause systemic endothelial dysfunction, which in turn will cause preeclampsia syndrome. Thus, the worsening of pregnancy outcomes that are in line with the increasing expression of miR-200a indicate that miR-200a plays a role in the pathogenesis of early-onset preeclampsia.

miR-200a was found in 41.9% of cases of late-onset preeclampsia, thus miR-200a may have a role in the etiopathogenesis of 41.9% of late-onset preeclampsia cases. It is assumed that there is a mild placentation defect in late-onset preeclampsia, and this minimal placentation defect does not interfere with the fulfillment of placental and fetal oxygenation. However, after pregnancy or near the end of pregnancy (34 weeks and beyond), placenta suffers from hypoxia due to minimal placentation defect and placental aging, accompanied by increased need of placental and fetal oxygenation. Thus, a late-onset preeclampsia appears.(20) On the other hand, high miR-200a has an anti-inflammatory effect on the vascular system (11), while increasing systemic (vascular) inflammatory response in pregnancy is a main etiopathogenesis of late-onset preeclampsia.(14) Due to

Table 2. Qualitative miR-200a expression, quantitative miR-200a expression, and NO level in EOPE and LOPE subjects.

Variables	Onset of Preeclampsia		*p*-value	OR
	EOPE* (n=31)	LOPE* (n=31)		
Qualitative miR-200a expression, n (%)				
Detected	23(74.2)	13(41.9)	0.010*[a]	3.98 (1.36-11.67)
Undetected	8(25.8)	18(58.1)		
Quantitative miR-200a expression (ng/mL), median (min-max)	36.75 (0.00-49.48)	0.00 (0.00-49.28)	0.048*[b]	
NO level (µmol/L), median (min-max)	23.75 (1.25-319.00)	106.00 (31.25-304.25)	0.027*[b]	

*Significant if $p<0.05$, [a]Tested with Chi-square test; [b]Tested with Mann Whitney-U test.

Table 3. NO level in subjects with detected and undetected miR-200 expression.

miR-200a Expression	Median (Min-Max) (µmol/L)	*p*- value
Yes	62.75 (0.25-319.00)	0.032*
No	132.25 (8.50-292.00)	

*Significant if $p<0.05$, tested with Mann Whitney-U test.

anti-inflammatory effect of miR-200a, it can be concluded that miR-200a suppresses/prevents the occurrence of late-onset preeclampsia.

In this study, the median serum NO levels were 23.75 nmol/mL in the early-onset preeclampsia group and 106.00 nmol/mL in the late-onset preeclampsia group. These two levels were significantly different. NO level in normal pregnancies was 35.1 nmol/mL (21), so NO level in early-onset preeclampsia was lower, while it was so high over normal pregnancies NO level in late-onset preeclampsia. NO is produced from the oxidation of l-arginine catalyzed by endothelial-nitric oxide synthase (eNOS) to produce NO and citrulline.(22) Any mechanism in early-onset preeclampsia leading to low eNOS production and/or activity may reduce NO production, resulting in low NO levels. It is hypothesized that silent information regulator-1 (SIRT1) found in the endothelium is a target gene of miR-200a. SIRT1 may upregulates eNOS activity and increases NO production. miR-200a regulates the SIRT1 gene by binding to the SIRT1 3'-UTR position, resulting in posttranscriptional inhibition (23), leading to no eNOS upregulation activity and decreased NO production.

Hypoxic placental increases ROS production and causes oxidative stress (4), and lead to suppresses eNOS activity or even damage it (uncoupling). The eNOS-uncoupling mechanism begins with the initial formation of superoxide and is then followed by the formation of peroxynitrite (ROS-induced ROS formation).(24) On the other hands, the hypoxic placenta releases proinflammatory factors (cytokines and various immune cells) into the maternal circulation.(4) eNOS uncoupling may also be manifested after being stimulated by various inflammatory mediators.(25) Endothelial cells and macrophages secrete arginase, which results in reduced l-arginine and thus lowering NO production. In addition, the redox status greatly affects the production of asymmetric dimethyl-L-arginine (ADMA). The role of ADMA is as an endogenous inhibitor of eNOS.(26)

NO level in late-onset preeclampsia was so high over normal pregnancy NO level. There is no satisfying theory can explain this phenomenon. Due to hormonal theory, it is supposed that estrogen hormone may activates eNOS function through rapid signaling mechanism leading to increase eNOS activation, and through longer term genomically regulated mechanism leading to increase mRNA eNOS and its protein. The end product of both mechanism is an increasing of NO production.(27) NO production is not merely in endothelial cells lining vascular, but it also been produced in perivascular adiposite tissue.(28) Regardless no theory to explain the phenomenon, it was realized that low NO levels was not a main cause of hypertension in late onset preeclampsia, but it was in early-onset preeclampsia. This point of view implicates that there should be a difference choice of first line treatment of hypertension between the two types of preeclampsia.

Table 3 shows that NO level in miR-200a group was significantly lower than that in non-miR-200a group. In accordance with the above discussion about NO level, the low NO production may be resulted from inhibition of eNOS upregulation by regulation SIRT1 gene, suppression of eNOS activity by ROS or by proinflammatory factors, reducing of l-arginine by inflammatory cell, and suppression of NO production by anti-angiogenic factors. All of these mechanisms are related to high miR-200a expression. So, it can be concluded that low level of NO associates with high miR-200a expression, or in other word, the effect of high miR-200a expression is not merely to causes impaired trophoblast proliferation, migration, and invasion, but leads to cause endothelial dysfunction too.

Conclusion

miR-200a was more expressed in EOPE compared to LOPE subjects, suggesting that it might have a significant role in pathomechanism of EOPE and can be considered as a significant marker in predicting EOPE. While NO level was significantly lower in EOPE whilst higher in LOPE subjects, showing that NO is not involved in EOPE and has a significant role in pathomechanism of LOPE only, and hence might be potential as marker to differentiate EOPE and LOPE.

Authors Contribution

HRK and Y were involved in the study conception. HRK perform the data acquisition, while HRK and H analyzed the data. HRK, Y, and J interpreted the results. All authors are invovled in the manuscript preparations and revisions.

References

1. Ives CW, Sinkey R, Rajapreyar I, Tita ATN, and Oparil S. Preeclampsia — pathophysiology and clinical presentations. J Am Coll Cardiol. 2020; 76(14): 1690–702.
2. Putri RWR, Prasmusinto D, Wibowo N, Irwinda R, Purwosunu Y, Saroyo YB. Higher trace elements and lower fatty acids levels in erythrocytes as predictors of preeclampsia. Indones Biomed J. 2024; 16(6): 560–71.
3. Shaw LJ, Patel K, Lala-Trindade A, Feltovich H, Vieira L, Kontorovich A, *et al.* Pathophysiology of preeclampsia-induced vascular dysfunction and implications for subclinical myocardial damage and heart failure. JACC Adv. 2024; 3(6): 100980. doi: 10.1016/j.jacadv.2024.100980.
4. Kornacki J, Olejniczak O, Sibiak R, Gutaj P, Wender-Ozegowska E. Pathophysiology of pre-eclampsia—two theories of the development of the disease. Int J Mol Sci. 2023; 25(1): 307. doi: 10.3390/ijms25010307.
5. Tomimatsu T, Mimura K, Endo M, Kumasawa K, Kimura T. Pathophysiology of preeclampsia: An angiogenic imbalance and long-lasting systemic vascular dysfunction. Hypertens Res. 2017; 40(4): 305–10.
6. Burton GJ, Redman CW, Roberts JM, and Moffett A. Pre-eclampsia: Pathophysiology and clinical implications. BMJ. 2019; 366: l2381. doi: 10.1136/bmj.l2381.
7. Giannubilo SR, Cecati M, Marzioni D, Ciavattini A. Circulating miRNAs and preeclampsia: From implantation to epigenetics. Int J Mol Sci. 2024; 25(3):1418. doi: 10.3390/ijms25031418.
8. Sumawan H, Giantari I, Mubarika S, Hadiati DR, Pradjatmo H. Exosomal miRNAs as potential biomarkers for preeclampsia: miR-1283 has the highest expression, while miR-152-3p has the lowest expression. Indones Biomed J. 2024; 16(4): 387–96.
9. Nelson N, Yusrawati, Serudji J, Jamsari, Amir A, Afriwardi, *et al.* Elevation of miR-210 expression and mean arterial pressure as early-onset pre-eclampsia biomarkers, while elevation of matrix metalloproteinase-2 as late-onset pre-eclampsia biomarker. Indones Biomed J. 2025; 17(2): 180–7.
10. He X, Ding D. High miR-200a-3p expression has high diagnostic values for hypertensive disorders complicating pregnancy and predicts adverse pregnancy outcomes. BMC Pregnancy Childbirth. 2022; 22(1): 490. doi: 10.1186/s12884-022-04785-x.
11. Wang C, Tsai P, Chen T, Tsai H, Kuo P, Su M. Elevated miR-200a and miR-141 inhibit endocrine gland-derived vascular endothelial growth factor expression and ciliogenesis in preeclampsia. J Physiol. 2019; 597(12): 3069–83.
12. Wu G, Jin Y, Cao Y, Li J. The diagnostic value and regulatory mechanism of miR-200a targeting ZEB1 in pregnancy-induced hypertension. Hypertens Pregnancy. 2020; 39(3): 243–51.
13. Nemecz M, Alexandru N, Tanko G. Role of microRNA in endothelial dysfunction and hypertension. Curr Hypertens Rep. 2016; 18(12): 87. doi: 10.1007/s11906-016-0696-8.
14. Redman CW. Early and late onset preeclampsia: Two sides of the same coin. Hypertens Pregnancy. 2017; 7: 58. doi: 10.1016/j.preghy.2016.10.011.
15. Shi C, Yang Y, Zhang L, Yu J, Qin S, Xu H, Gao Y. MiR-200a-3p promoted the malignant behaviors of ovarian cancer cells through regulating PCDH9. Onco Targets Ther. 2019; 12: 8329–38.
16. Su MT, Tsai PY, Wang CY, Tsai HL, Kuo PL. Aspirin facilitates trophoblast invasion and epithelial-mesenchymal transition by regulating the miR-200-ZEB1 axis in preeclampsia. Biomed Pharmacother. 2021; 139: 111591. doi: 10.1016/j.biopha.2021.111591.
17. Saha S, Choudhury J, Ain R. MicroRNA-141-3p and miR-200a-3p regulate insulin-like growth factor 2 during mouse placental development. Mol Cell Endocrinol. 2015: 414: 186–93.
18. Puspasari A, Enis RN, Herlambang. Genetic variant of vascular endothelial growth factor (VEGF)-A rs699947 is associated with preeclampsia. Mol Cell Biomed Sci. 2022; 6(2): 70–6.
19. Serudji J, Basyir V, Fadhillah T. Differentiating material angiogenesis factors between early and late onset preeclampsia: Higher sFlt-1 in early onset preclampsia, lower PlGF and higher sFlt-1/PlGF ratio in late-onset preeclampsia. Mol Cell Biomed Sci. 2024; 8(3): 154–58.
20. Staff AC. The two-stage placental model of preeclampsia: An update. J Reprod Immunol. 2019; 134–135: 1–10. doi: 10.1016/j.jri.2019.07.004.
21. Hodzic J, Izetbegivic S, Muracevic B, Iriskic R, Jovic HS. Nitric oxide biosynthesis during normal pregnancy and pregnancy complicated by preeclampsia. Med Glas. 2017; 14(2): 211–7.
22. Degjoni A, Campolo F, Stefanini L, Venneri MA. The NO/cGMP/PKG pathway in platelets: The therapeutic potential of PDE5 inhibitors in platelet disorders. J Thromb Haemost. 2022; 20(11): 2465–74.
23. Pan F, Qiu X, Yu W, Zhang Q, Chen Q, Zhang C, *et al.* MicroRNA-200a is up-regulated in aged rats with erectile dysfunction and could attenuate endothelial function via SIRT1 inhibition. Asian J Androl. 2016; 18(1): 74–9.
24. Daiber A, Xia N, Steven S, Oelze M, Hanf A, Kröller-Schön S, *et al.* New therapeutic implications of endothelial nitric oxide synthase (eNOS) function/dysfunction in cardiovascular disease. Int J Mol Sci. 2019; 20(1): 187. doi: 10.3390/ijms20010187.
25. Theofilis P, Sagris M, Oikonomou E, Antonopoulos AS, Siasos G, Tsioufis C, *et al.* Inflammatory mechanisms contributing to endothelial dysfunction. Biomedicines. 2021; 9(7): 781. doi: 10.3390/biomedicines9070781.
26. Cyr AR, Huckaby LV, Shiva SS, Zuckerbraun BS. Nitric oxide and endothelial dysfunction. Crit Care Clin. 2020; 36(2): 307–21.
27. Duckles SP, Miller VM. Hormonal modulation of endothelial NO production. Pflugers Arch. 2010; 459(6): 841–51.
28. Nava E, Llorens S. The local regulation of vascular function: From an inside-outside to an outside-inside model. Front Physiol. 2019; 10: 729. doi: 10.3389/fphys.2019.00729.