**Medical and Health Journal**
Volume 5, Issue 1, 2025, pp. 214-224
e-ISSN: 2807-3541
Open Access: https://jos.unsoed.ac.id/index.php/mhj

# Microrna Profile of Plasma Exosomes by Nanostrings in Early Onset Compared Late Onset Preeclampsia: Preliminary Study

**Herman Sumawan[1*], Ifrinda Giantari[2], Sofia Mubarika[3], Diah Rumekti Hadiati[4], Heru Pradjatmo[4]**

[1]Department of Obstetrics and Gynecology, Jenderal Soedirman University, Purwokerto, Indonesia
[2]Department of Obstetrics and Gynecology, RAA Soewondo Hospital, Pati, Indonesia
[3]Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Yogyakarta, Indonesia
[4]Department of Obstetrics and Gynecology, Gadjah Mada University, Sardjito Hospital Yogyakarta, Indonesia

**ARTICLE INFO**

**Article history:**
Received August 24, 2025
Revised August 25, 2025
Accepted August 26, 2025
Available online August 27, 2025

**Keywords:**
Exosomes, miRNAs, early onset preeclampsia, late onset preeclampsia

***ABSTRACT***

*Research on miRNA biomarkers in preeclampsia as part of screening, diagnosis, and prognosis has been widely conducted, but the results show contradictory results and vary based on the type of preeclampsia. This study aims to compare the profile of plasma exosome miRNA in early onset compared late onset as a preliminary study to identify the miRNA profile of preeclampsia patients in Indonesia. The study was conducted at Margono Hospital,Indonesia using plasma exosomes samples of three patients with early-onset preeclampsia and three patients with late-onset preeclampsia and processed with NanoStrings. KEGG was used to identify preeclampsia pathophysiological pathways by bioinformatic analysis of DIANA-miRPath v3.0 and microT-CDS v5.0. The results showed that the characteristics of parity, hemoglobin, systolic and diastolic blood pressure, proteinuria and BMI did not differ between EOPE and LOPE. Significantly different variables were the age of the EOPE (28 ± 5.29) vs LOPE (38.67 ± 2.06 mmHg), pregnancy weight gain (10.0 vs 15.33), and fetal weight in EOPE (1550 ± 132 g) vs LOPE (2693 ± 716 g). The results showed that the 24 miRNAs differed significantly. The three highest expression miRNAs in the EOPE group were miR-196b-5p, miR-190a-5p, and miR-515-3p. In contrast, the three lowest expression miRNAs are miR-3179, miR-181a-5p, and miR-15b-5p. Pathway analysis of the upregulated miRNA involved the ErbB signalling pathway, Proteoglycan in cancer, and Lysin degradation. Downregulated miRNA targets involved in the HIPPO signalling pathway, fatty acid biosynthesis, and TGF-β signalling pathway. Conclusions: The preliminary study results indicated significant differences in miRNA expression, suggesting that EOPE is influenced by aggressive cellular signaling and metabolic dysregulation, while LOPE is more linked to the disruption of growth-inhibiting pathways and fatty acid metabolism. These unique miRNAs establish a robust foundation for subsequent validation studies utilizing bigger samples as a prospective biomarker panel.*

## 1. INTRODUCTION

Preeclampsia impacts 2–8% of pregnancies globally. Approximately 46,000 maternal fatalities occur annually owing to pre-eclampsia, alongside roughly 500,000 fetal or neonatal fatalities. Pre-eclampsia and eclampsia account for roughly 10% of maternal fatalities in Asia and Africa and 25% in Latin America (Cresswell et al., 2025). In Indonesia, preeclampsia is the leading cause of death: in 2022, the Ministry of Health reported that 801 of the total 3,572 cases of maternal death (22.42%) were caused by hypertension during pregnancy (Kemenkes, 2023). Preeclampsia can be early onset (EOPE) or late onset (LOPE), depending on when clinical symptoms appear (before or after the 34th week of pregnancy). In addition, EOPE has a more severe course and accounts for 5–20% of all kinds of preeclampsia. Adverse outcomes for the fetus are associated with chronic hypoxia and a high frequency of developmental delay, as well as complications in the fetus due to prematurity, including respiratory distress syndrome, infectious and inflammatory diseases, intraventricular hemorrhages, cerebral palsy, cognitive retardation, autism, psychomotor and behavioral disorders, and/or learning disabilities (Timofeeva et al., 2023).

*Corresponding author
E-mail addresses: h.sumawan@gmail.com (Herman Sumawan)

Single-stranded, medium- to long-length (20–24 nt) noncoding RNAs known as microRNAs (miRNAs) regulate gene expression. MiRNAs play a role in regulating various physiological processes, and their dysregulation has been linked to the development of several diseases, including preeclampsia (Xu et al., 2021; Cirkovic et al., 2021). The expression levels of miRNAs in placental tissue increase during pregnancy and throughout placental development. miRNAs are crucial for the biological functions of trophoblasts, including trophoblast differentiation, invasion, migration, proliferation, apoptosis, angiogenesis, cellular metabolism, lipid metabolism, nervous system development, and immune and inflammatory responses (Gu Fu et al., 2022; Jairajpuri et al., 2021; Zhou et al., 2020). The abnormal process occurring in placental development due to aberrant miRNA expression results in impaired differentiation and apoptosis of cytotrophoblasts, incomplete invasion of spiral arteries, and reduced blood flow to and from the placenta. Trophoblast necrosis releases cell fragments into the maternal bloodstream, subsequently triggering a systemic immunological response and oxidative stress in the placenta. Thus, any dysregulation of miRNAs can affect the normal function of target genes in the placenta and in other systems (Oh et al., 2020).

Exosomes, microvesicles, and apoptotic bodies are the mechanisms through which miRNAs in the placenta are released into the bloodstream. These miRNAs have been identified in the plasma and blood of mothers (Salomon et al., 2017). Plasma miRNAs are beneficial for detecting pathological changes in diseases due to their stability, which remains unaffected by RNase, pH, and even incubation temperature (Sourvinou, Markou, and Lianidou, 2013). Consequently, identifying specific plasma miRNAs may help elucidate the pathophysiology of different types of preeclampsia and could potentially lead to the development of biomarkers for diagnosing and monitoring the severity of the condition. Next-generation sequencing, microarrays, and NanoStrings have been used in various studies to explore miRNAs in preeclampsia (Pillay et al., 2019). However, these studies have produced a range of findings regarding miRNA expression, suggesting that the pathophysiology of preeclampsia may differ across populations. This variability may be linked to individual patient characteristics, lifestyle, ethnicity, race, and different environmental exposures (Zhong, Zhu, and Ding, 2019).

Our objective was to utilize bioinformatic analysis to investigate the miRNA profile in order to ascertain which miRNAs were associated with the type of preeclampsia and which pathophysiological pathways were predominantly involved in the occurrence of preeclampsia. This information may serve as a foundation for the further investigation of preeclampsia, to obtain biomarkers for screening, monitoring, and differentiating types of preeclampsia as well as providing scientific information on the miRNA profile of early onset and late-onset preeclampsia in Indonesia, which has not existed until now. The miRNA profiles that were identified were analyzed using NanoStrings technology. This technology simultaneously identifies over 800 miRNAs and exhibits exceptional digital precision and sensitivity. NanoString technology offers a precise representation of miRNA expression in a population, as it does not necessitate amplification, such as if using other technologies, namely microarray and next-generation sequencing (Pillay et al., 2019)

## 2. METHOD

This study received institutional, ethical, and regulatory approval from the Medical and Health Sciences Faculty of Gadjah Mada University. Patients were recruited from Margono Hospital, Purwokerto, Banyumas, Central Java, with informed consent, as required by the Declaration of Helsinki.

### Study groups

We enrolled consecutive pregnant women (at 20–40 weeks of gestation) and Consecutive expectant women (between 20 and 40 weeks of gestation) were enrolled and subsequently divided into two groups: three participants with EOPE and three with LOPE. Preeclampsia was defined as the onset of hypertension (systolic blood pressure [BP] ≥ 140 mmHg and diastolic BP ≥ 90 mmHg at two measurements taken at an interval of at least 4 h) and proteinuria (i.e., ≥ 30

mg/mol protein:creatinine ratio, ≥ 300 mg/24 hours, or two or more positive dipstick results) after the 20th week of gestation in a patient who was previously normotensive. Severe preeclampsia was defined as the presence of at least one of the following signs: pulmonary oedema, renal abnormalities (serum creatinine > 1 mg/dL), uteroplacental dysfunction, cerebral symptoms (such as persistent headaches and neurological symptoms), visual disturbances, or abnormal liver function, enzymes, or platelet counts < 150,000/mL. Patients who did not have proteinuria but met one of the aforementioned criteria and had hypertension (BP ≥ 140/90) were classified as having severe preeclampsia. Patients are classified as EOPE when preeclampsia develops prior to 34 weeks of gestation and as LOPE when it develops after 34 weeks (Magee et al., 2022).

Patients with autoimmune diseases, diabetes mellitus, renal diseases, cardiovascular diseases, or infectious diseases were excluded from this study, as were those with multiple pregnancies. When the patient came the hospital, vacutainer tubes (EDTA) were employed to collect blood. Plasma was obtained by centrifuging the blood at 3,500 × g for 10 minutes. The samples were subsequently preserved at −80 °C for exosome extraction.

**Exosome isolation**

In order to isolate exosomes, extract total RNA, and separate RNA from proteins, we employed a Total Exosome Isolation Kit (Invitrogen, Waltham, MA, USA) and a Total Exosome RNA and Protein Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA), adhering to the manufacturer's procedures. Sample preparation entailed the centrifugation of the plasma sample at 2,000 × g for 20 minutes at room temperature to remove cells and detritus following defrosting. The particle was not disturbed during the transfer of the clear plasma-containing supernatant to a new tube. Finally, the containers were centrifuged at 10,000 × g for 20 minutes at room temperature to remove any debris. The supernatant containing the purified plasma was transferred to a new tube without disturbing the pellet, and isolation was continued. The clarified plasma and 0.5 L 1× PBS were combined in the tube and vigorously mixed using a vortex mixer. Subsequently, we introduced 0.05 volumes of proteinase K to the sample. The vessel was incubated at 37°C for 10 minutes following the vortexing of the sample. Subsequently, we introduced 0.2 of the exosome precipitation reagent to the samples (total volume = plasma + PBS) and thoroughly mixed the samples by vortexing or inverting. Finally, the samples were incubated at 2–8 °C for 30 minutes. The samples were centrifuged at 10,000 × g for 5 minutes at ambient temperature following incubation. The supernatant was subsequently discarded and the particle containing the exosomes was used for further extraction. The supernatant was removed using a pipette. The exosomal RNA was isolated using the Total Exosomal Protein and RNA Separation Kit. The exosomal particle was resuspended in the exosome resuspension buffer in accordance with the manufacturer's instructions. The total RNA concentration was determined using a NanoDrop 2000 spectrophotometer from Thermo Fisher Scientific.

**MiRNA testing with the NanoString nCounter system**

Exosomal RNA was analysed in the two sample groups utilising a NanoString nCounter SPRINT Profiler (NanoString Technologies, Inc., Seattle, WA, USA). The Quick-RNA Miniprep Kit (Zymo Research) was utilised for total RNA extraction. Genomic DNA contamination in RNA was eliminated through the application of DNase and RNAClean XP (Beckman Coulter Diagnostics, Brea, CA, USA). The RNA sample concentrations were evaluated using a NanoDrop (Thermo Scientific) and TapeStation (Agilent Technologies, Inc., Santa Clara, CA, USA). The nCounter Digital Analyser documented the reporter probe counts for each sample, and nSolver Software v4.0 (NanoString Technologies, Inc.) along with the ROSALIND platform (https://www.rosalind.bio/) were utilised for subsequent analysis. Prior to data normalisation, the following solver parameters were employed to assess the nCounter data imaging quality control metrics: binding density, imaging, positive control limit of detection, and positive control linearity. The binding densities of the samples ranged from 0.13 to 0.15, whereas the optimal range for the nCounter SPRINT system was 0.1 to 1.8 spots per square micron. The normalisation factor altered the variations in the quality and quantity of the analyte across the samples. Upon considering various

degradation states and eliminating input variation through normalisation, the acceptable values are confined to the default range of 0.1–10.

**Statistical Analysis**

Data and graphics were analysed using nSolver Analysis Software v4.0.7 (NanoString Technologies, Inc.). ROSALIND was utilised to calculate the fold changes and p-values through the t-test method for comparative analysis. The Benjamini–Hochberg method was employed to calculate the adjusted p-value and assess the false discovery rate (FDR). The comparison of miRNA ratios between the groups was conducted using nSolver analytic software and the ROSALIND platform. The criteria for selecting miRNA ratios included a fold change of at least 1.5 and a false discovery rate of 0.05 or lower on the log2 scale. A comprehensive analysis of the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways was conducted. Analyses were conducted utilising the Descriptive Intermediate Attributed Notation for Ada (DIANA)–miRPath (v3.0) and microT-CDS (v5.0) algorithms, with a gene union interaction p-value threshold set at < 0.05 and a microT threshold of 0.8. All graphics were created and analysed using GraphPad Prism 9 (GraphPad, Inc., La Jolla, CA, USA).

## 3. RESULT AND DISCUSSION

### Result

### Clinical characteristics of participants

The comparison of the characteristics of participants in the EOPE and LOPE groups showed that parity, BMI, blood pressure, hemoglobin, and proteinuria did not differ significantly (Table 1). As expected, gestational age was lower in participants with EOPE than in those with LOPE *($p$ < 0.05)*. Age, gestational weight gain, and fetal weight were higher in those with LOPE than in those with EOPE (both $p < 0.05$). The concentration of exosome RNA did not differ between EOPE and LOPE, and the amount was in accordance with the previous study.

Table 1. Clinical characteristics of participants [*significant differences ($p < 0.05$)].

| | EOPE | LOPE | p-value |
|---|---|---|---|
| Age (years)* | 28.00(± 5.29) | 38.67(± 2.06) | 0.031 |
| Gestational age (weeks)* | 32.00(± 1,73) | 37.00(± 2.00) | 0.031 |
| Parity | 1.0 (± 1.0) | 2.0 (± 1.0) | 0.288 |
| BMI (kg/m$^2$) | 25.55 (± 0.67) | 32.12 (± 4.74) | 0.076 |
| Gestational weight gain (kg)* | 10 (± 1.0) | 15.33(± 2.51) | 0.02 |
| Systolic BP (mmHg) | 156.00 (± 13.52) | 172.00 (± 24.24) | 0.375 |
| Diastolic BP (mmHg) | 99.33 (± 9.01) | 98.33 (± 10.40) | 0.906 |
| Proteinuria (gr/dl) | 333.33 (± 288.67) | 108.33 (± 72.16) | 0.260 |
| Hemoglobin (gr/dl) | 12.13 (± 3.32) | 10.77 (± 1.12) | 0.537 |
| Fetal weight* (gram) | 1550 (± 132.28) | 2693 (± 716) | 0.05 |
| RNA exosome concentration (ng/µL) | 12.47 | 11.78 | |

BP, blood pressure; BMI, body mass index

Data are presented as mean (± standard deviation).

The heatmap image in figure 1 shows miRNAs between early-onset and slow-onset preeclampsia, where there are 12 miRNAs that are increased in expression and 12 miRNAs that decrease in early onset preeclampsia compared to late-onset preeclampsia. Meanwhile, the highest fold change of 3 miRNA expression in the upregulated group was miR-196b-5p, miR-190a-5p, and miR-515-3p. In the downregulation group, miR-3179, miR-181a-5p, and miR-15b-5p were the miRs with the lowest expression (Figure 2).

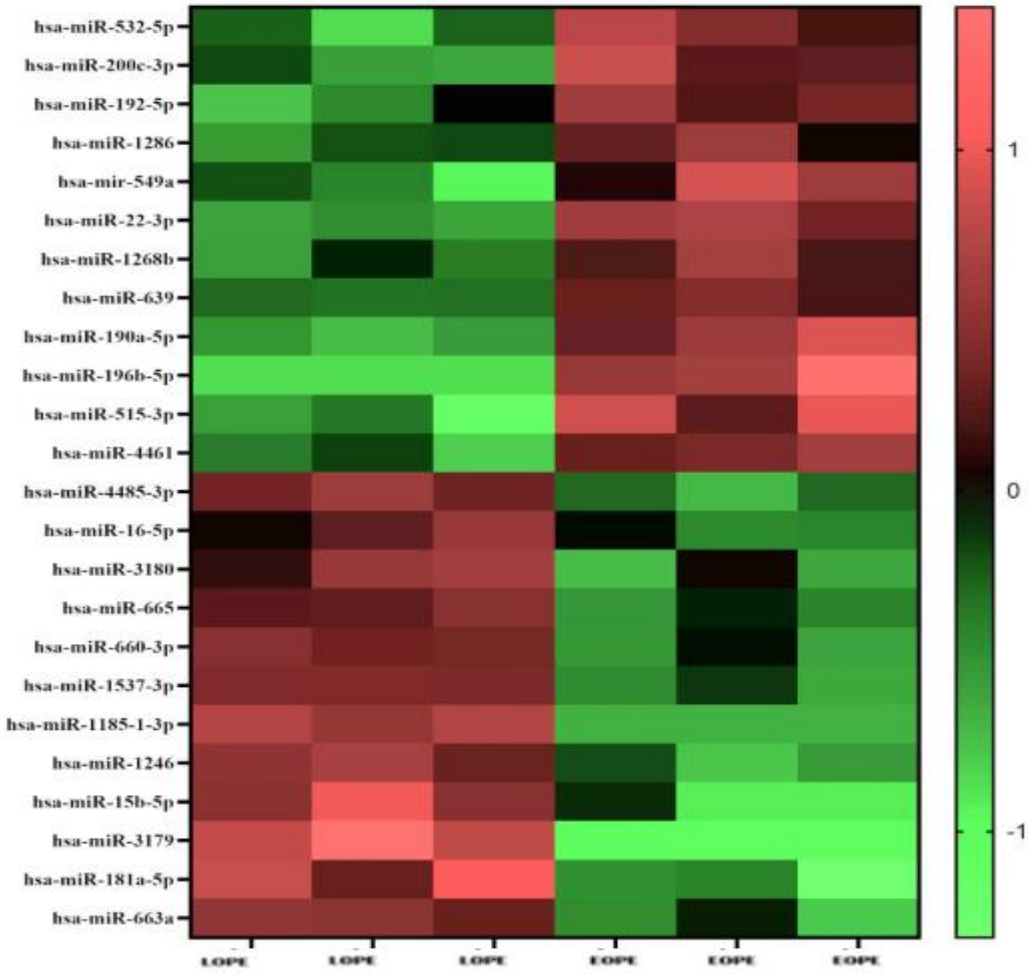


Figure 1. Heatmap comparison of miRNA expression between preeclampsia early onset (EOPE) and late onset (LOPE). Red indicates upregulation, and green indicates downregulation.

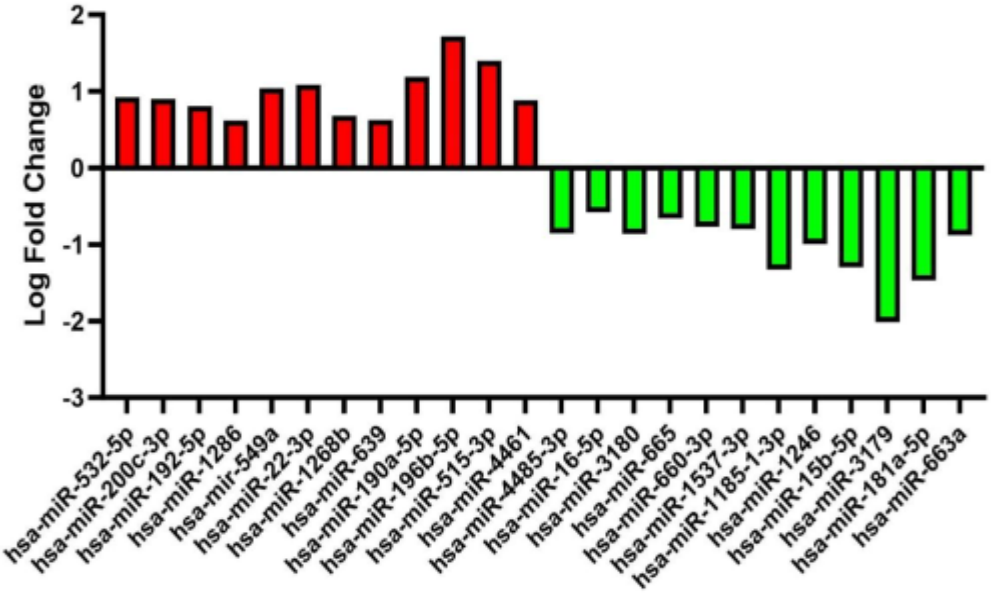


Figure 2. Log Fold Change of miRNA expression comparing early onset preeclampsia (EOPE) to late onset preeclampsia (LOPE). Red indicates upregulation, while green indicates downregulation.

The analysis of the enrichment pathway on predicted gene targets from the three upregulated miRNAs (miR-196b-5p, -190a-5p, and -515-3p) revealed significant involvement in several important pathways (Figure 3). These include the ErbB signaling pathway, which plays a crucial role in cell proliferation, differentiation, and migration. Dysregulation of this pathway is associated with the abnormal invasive behavior of trophoblasts. Additionally, the proteoglycans in cancer pathway modulates various cellular processes, including adhesion, proliferation, and angiogenesis, thereby connecting the microenvironment and cell signaling. The lysine degradation pathway is linked to energy metabolism and cellular homeostasis. Disruptions in amino acid metabolism have been implicated in endothelial dysfunction.

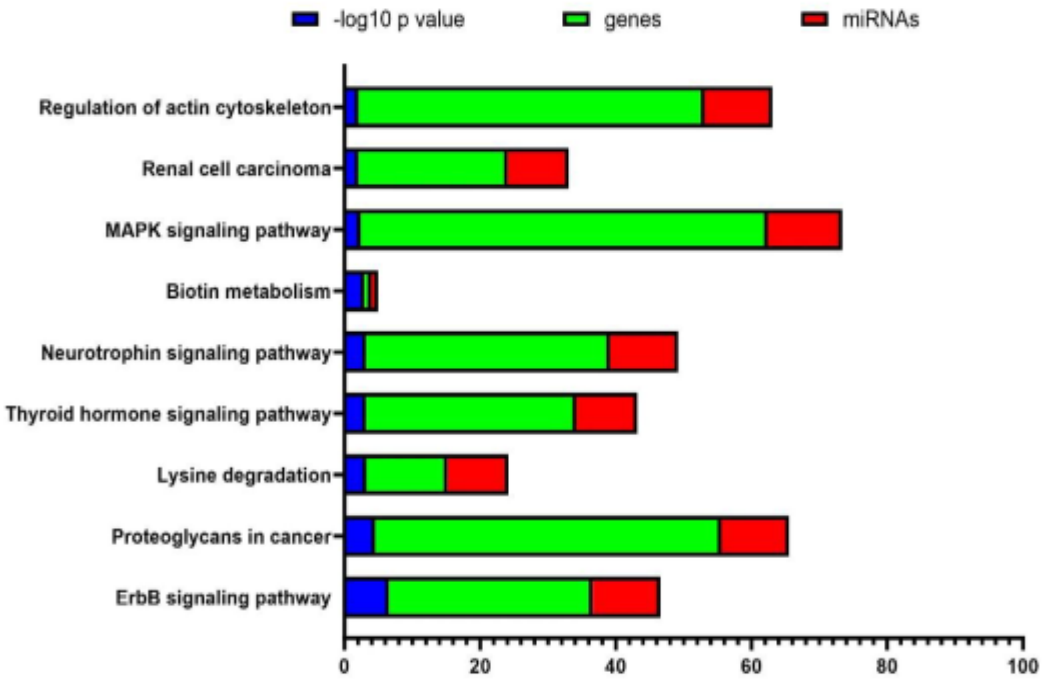


Figure 3**.** KEGG pathway upregulated miRNA expression between preeclampsia early onset (EOPE) and late onset (LOPE). Red indicates the number of interaction miRNAs, green indicates the number of gene targets, and blue indicates significant value (-log10 p-value).

KEGG pathway analysis of the downregulated miRNA group showed that the most significant preeclampsia-related pathways were the HIPPO signalling pathway, fatty acid biosynthesis, and the TGF-b signalling pathway (Figure 4). Analysis of the enrichment pathway in the predicted gene target of the three downregulated miRNAs (miR-3179, -181a-5p, -15b-5p) revealed significant enrichment in a very different pathway, the HIPPO signalling pathway, where this pathway is key to controlling organ size and cell proliferation by inhibiting overgrowth. Suppression of this pathway can lead to uncontrolled cell proliferation. The metabolic pathway plays a crucial role in energy production and cell membrane synthesis during fatty acid biosynthesis. Dysregulation of lipid metabolism is a known feature of PE pathogenesis. The TGF-β signaling pathway has a complex dual role in pregnancy, promoting trophoblast invasion and also having immunosuppressive effects. Disruption of this pathway can result in superficial implantation.

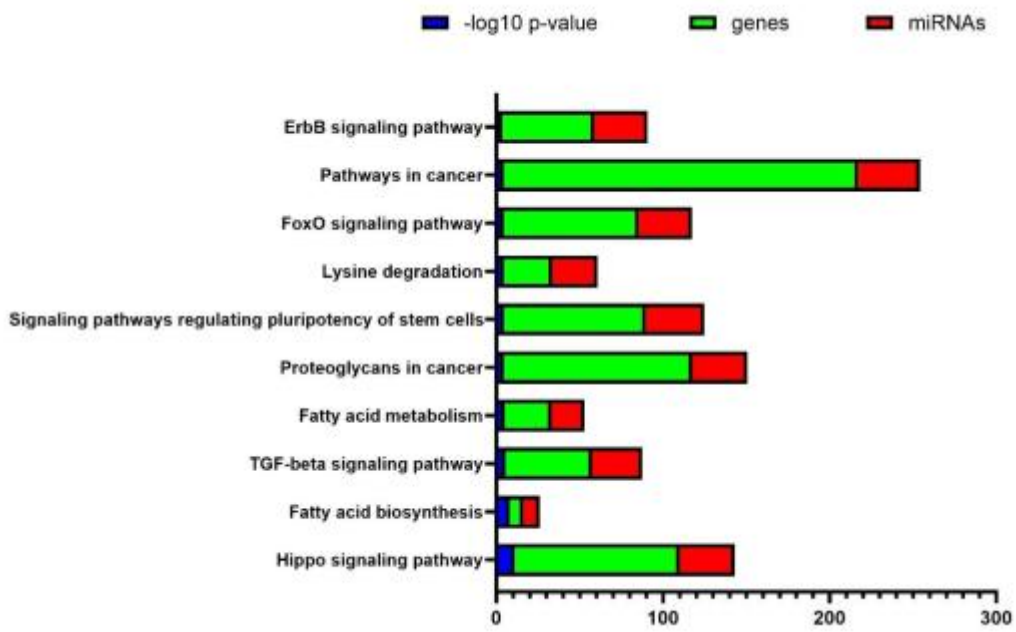


Figure 4. KEGG pathway downregulated miRNA expression between preeclampsia early onset (EOPE) and late onset (LOPE). Red indicates the number of interaction miRNAs, green indicates the number of gene targets, and blue indicates significant value (-log10 p-value).

## Discussion

In this study, we compared the miRNA profile of plasma exosomes from individuals with EOPE and those with LOPE to identify a biomarker related to its pathophysiology. In the sample characteristics, age and weight gain during pregnancy were significantly different between EOPE and LOPE. This is in line with previous cohort research where excess body lift occurred more in LOPE than in EOPE (Hutcheon et al., 2018). Meanwhile, the age variable in the study of the population in Surakarta did not differ significantly between the two groups (Damayanti S, Sulistyowati S, and Probandari, 2019).

We identified 12 downregulated and 12 upregulated miRNAs in women with EOPE compared to LOPE. All three upregulated miRNAs (miR-196b-5p, miR-190a-5p, and miR-515-3p) have previously been associated with proliferation, invasiveness, and metastasis processes in cancer research (Xin et al., 2020). Our findings suggest that this "cancer-like" pattern may also be present in EOPE. miR-196b-5p is known to target tumor suppressor genes and may increase cell proliferation (Luo, Sun, and Sun, 2019). Another study shows the same result: miR-196b-5p is involved in preeclampsia (Bao et al., 2022). The enrichment of the ErbB and proteoglycan pathways in cancer derived from this miRNA target reinforces the hypothesis that EO-PE may involve trophoblasts that have an uncontrollable and disrupted invasive phenotype, but unlike metastatic cancers, the invasion becomes superficial and inadequate, leading to poor spiral artery remodeling. This result is consistent with a previous study showing exomiRNAs in preeclamptic maternal circulation are connected to biological processes involved in cancer biology. This is consistent with the current notion that placental trophoblast and cancer cells can create a milieu that promotes immunologic privilege and angiogenesis via molecular pathways that govern hyper-proliferation, invasion, angiogenesis, and immunoevasion (Pillay et al., 2019).

Although the conventional lysine degradation pathways (through saccharopine or pipecolic acid) are not directly associated with preeclampsia, studies suggest that alterations in lysine, particularly lysine acetylation, play a significant role in the condition. The downregulation of lysyl oxidase (LOX), a lysine-modifying enzyme, along with changes in the lysine-specific demethylase 5C (KDM5C) pathway, are associated with the development of preeclampsia. This evidence indicates that lysine metabolism and modifications play a significant role in the pathogenesis of the condition (Shi et al., 2022). Conversely, the downregulation of miR-181a-5p

and miR-15b-5p, which generally function as tumor suppressors by modulating cell cycling and apoptosis in EOPE, indicates a loss of mechanisms that control cell growth. The downregulation of miR-15b-5p, recognized as a negative regulator of angiogenesis, may represent an insufficient compensatory mechanism to enhance angiogenesis in an anti-angiogenic context. The reduced expression of miR-15b-5p aligns with findings from earlier research (Jairajpuri et al., 2017; Ura et al., 2014). MiR-15b-5p promotes the production of proinflammatory cytokines essential for the function of human placental immune cells by inhibiting apelin signalling (Yadava et al., 2021). This miRNA plays a role in various immunological diseases and induces cell cycle arrest in cervical cancer and glioma cell lines through the targeting of cyclin E1 (Mayor-Lynn et al., 2011). Prior research indicates that miR-15b suppresses endothelial cell tube formation and trophoblast invasion through the downregulation of AGO2 expression. AGO2 is exclusively present in cytotrophoblasts and endothelial cells of the placenta, which play a role in trophoblast invasion and the development of the endothelial tube (Yang et al., 2016).

This aligns with the HIPPO enrichment pathway, where suppression of the HIPPO pathway can result in excessive yet poorly differentiated trophoblast proliferation, contributing to abnormal placentation. The HIPPO signaling pathway was the most significant in downregulating miRNAs. The pathway is associated with the TGF-β and WNT signaling pathways, which were also significant in this study. TAZ and YAP regulate the HIPPO signaling pathway. Consequently, precise management of the quantities and locations of these elements is essential for early developmental processes, as well as for tissue stability, healing, and regrowth (Varelas 2014). YAP regulates CDX2, influencing trophoblast invasion and apoptosis in preeclampsia (Sun et al., 2018; Liu et al., 2020). The findings align with previous research indicating that the HIPPO signalling pathway is a significant pathway for miRNA downregulation in preeclampsia (Pillay, 2019).

Our research identifies another mechanism: the role of lipid metabolism in the regulation of fatty acid production. Fatty acids are essential for cell proliferation, cell signaling, and the development of important structural and functional characteristics in the fetoplacental unit. Fatty acids play a role in the initial phases of placental development by regulating angiogenesis during the first trimester (Duttarory and Basak, 2022). Lipids influence preeclampsia by controlling vascular and trophoblast activity. Recent evidence suggests that in preeclampsia, miRNAs have a role in adipogenesis, where disruption of adiponectin can prevent AMPK functioning and produce mitochondrial malfunction and insulin resistance (Hu et al., 2022).

The main limitation of this study is the small sample size (n=3 per group), which limits the statistical strength and generalization of the findings. However, the consistency and magnitude of the fold change, as well as the use of nanostring technology, provide confidence for further validation studies. Future research should validate these 6 miRNA signatures in a larger independent cohort using methods such as RT-qPCR. In vitro and in vivo functional studies are also needed to validate specific gene targets and prove a causal relationship between these miRNAs and identified pathways.

## 4. CONCLUSION

Although preliminary, the data from this study provide valuable molecular insights into the pathogenic differences between EOPE and LOPE. The identified signature miRNAs—consisting of miR-196b-5p, miR-190a-5p, miR-515-3p, miR-3179, miR-181a-5p, and miR-15b-5p—have the potential to be developed into a non-invasive biomarker panel that can distinguish the two PE subtypes early. In addition, bioinformatics analysis leads to a new hypothesis that EOPE is not only about "less invasiveness" but may also involve "uncontrolled and irregular invasiveness," which is reflected by the activation of cancer-like pathways and suppression of growth-inhibiting pathways. This insight could pave the way for targeted therapeutic strategies that specifically address the unique biological behaviors of each subtype. Further research is needed to validate these findings and explore the clinical implications of such a biomarker panel in improving patient outcomes.

## 5. ACKNOWLEDGE

**Ethics approval and informed consent:** The study protocol was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, with ethical committee reference number KE-FK/1049/EC/2021. All participants gave informed consent for this study.

**Consent for publication:** Not applicable

**Competing interests:** The authors declare that they have no competing interests.

**Funding information:** This study was supported by the Indonesia Endowment Fund for Education Agency. The funder has no role in the conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript.

**Authors' contributions:** HS, SF, DRH, and HP contributed to the design and conceptualisation of the manuscript. HS and IG contributed to the implementation and analysis of results. HS contributed to the data collection and wrote the first draft of the manuscript. All the authors have read and approved the final manuscript.

## 6. REFERENCES

Bao S, Zhou T, Yan C, Bao J, Yang F, Chao S, Zhou M, Xu Z. 2022. A blood-based miRNA signature for early non-invasive diagnosis of preeclampsia. *BMC Med.* Sep 13;20(1):303

Cirkovic A, Stanisavljevic D, Milin-Lazovic J, Rajovic N, Pavlovic V, Milicevic O, et al. 2021. Preeclamptic Women Have Disrupted Placental microRNA Expression at the Time of Preeclampsia Diagnosis: Meta-Analysis. *Front Bioeng Biotechno*l 9 https://www.frontiersin.org/articles/10.3389/fbioe.2021.782845

Cresswell JA, Alexander M, Chong MYC, Link HM, Pejchinovska M, Gazeley U, Ahmed SMA, Chou D, Moller AB, Simpson D, Alkema L, Villanueva G, Sguassero Y, Tunçalp Ö, Long Q, Xiao S, Say L. 2025. Global and regional causes of maternal deaths 2009-20: a WHO systematic analysis. *Lancet Glob Health*. Apr;13(4):e626-e634. doi: 10.1016/S2214-109X(24)00560-6.

Damayanti S, Sulistyowati S, and Probandari AN, 2019. Maternal Characteristics and the Effects of Early and Late-onset Types of Preeclampsia on Maternal and Perinatal Complications. *Indonesian Journal of Medicine*,Vol 4 No 4

Duttaroy AK, Basak S. 2022. Maternal Fatty Acid Metabolism in Pregnancy and Its Consequences in the Feto-Placental Development. *Front Physio*l 12. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2021.787848

Gu Fu,Lu D, Zhang L. 2022. MicroRNA-30a contributes to pre-eclampsia through regulating the proliferation, apoptosis, and angiogenesis modulation potential of mesenchymal stem cells by targeting AVEN. *Bioengineered*. 13(4):8724–34.

Hu M, Li J, Baker PN, Tong C. Revisiting preeclampsia: a metabolic disorder of the placenta. *FEBS J* . 2022 Jan 1;289(2):336–54. https://doi.org/10.1111/febs.15745

Hutcheon JA, Stephansson O, Cnattingius S, Bodnar LM, Wikström AK, Johansson K. 2018. Pregnancy Weight Gain Before Diagnosis and Risk of Preeclampsia: A Population-Based Cohort Study in Nulliparous Women. *Hypertension*. Aug;72(2):433-441

Inggas, Made Agus M., et al. Biokimia, Biologi Molekuler dan Aplikasinya Dalam Bidang Kesehatan. Mafy Media Literasi Indonesia Publisher, 2024, doi:10.1112/kakinaan1132.

Jairajpuri DS, Malalla ZH, Mahmood N, Almawi WY. Circulating microRNA expression as predictor of preeclampsia and its severity. *Gene*. 2017;627:543–8. https://www.sciencedirect.com/science/article/pii/S037811191730522X

Jairajpuri DS, Malalla ZH, Sarray S, Mahmood N. 2021. Analysis of differential expression of hypoxia-inducible microRNA-210 gene targets in mild and severe preeclamptic patients.

*Noncoding RNA Res* 6(1):51–7. https://www.sciencedirect.com/science/article/pii/S2468054021000056

Kementerian Kesehatan Republik Indonesia. 2023. Profil Kesehatan Indonesia Tahun 2022. Jakarta.

Liu R, Wei C, Ma Q, Wang W. 2020. Hippo-YAP1 signaling pathway and severe preeclampsia (sPE) in the Chinese population. *Pregnancy Hypertens* 19:1–10. https://www.sciencedirect.com/science/article/pii/S2210778919304519

Luo M, Sun G, Sun JW. 2019. MiR-196b affects the progression and prognosis of human LSCC through targeting PCDH-17. *Auris Nasus Larynx*. Aug;46(4):583-592.

Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, Kenny LC, McCarthy F, Myers J, Poon LC, Rana S, Saito S, Staff AC, Tsigas E, von Dadelszen P. 2022. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. *Pregnancy Hypertens*. Mar;27:148-169. doi: 10.1016/j.preghy.2021.09.008. Epub 2021 Oct 9. PMID: 35066406.

Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. 2011. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. *Reproductive Sciences*. Jan;18(1):46–56.

Oh SY, Hwang JR, Choi M, Kim YM, Kim JS, Suh YL, Choi SJ, Roh CR. 2020. Autophagy regulates trophoblast invasion by targeting NF-κB activity. *Sci Rep*. Aug 20;10(1):14033. doi: 10.1038/s41598-020-70959-2.

Pillay P, Vatish M, Duarte R, Moodley J, Mackraj I. 2019. Exosomal microRNA profiling in early and late onset preeclamptic pregnant women reflects pathophysiology. *Int J Nanomedicine*. 14:5637–57.

Salomon C, Guanzon D, Scholz-Romero K, Longo S, Correa P, Illanes SE, et al. 2017. Placental Exosomes as Early Biomarker of Preeclampsia: Potential Role of Exosomal MicroRNAs Across Gestation. *J Clin Endocrinol Metab*. Sep 1;102(9):3182–94. https://doi.org/10.1210/jc.2017-00672

Shi XF, Zhang Z, Wu HY, Wang Y, Chang AM, Gao JJ, Liu K, Song WY, Wang L, Wang HP. 2022. Lysine (K)-specific demethylase 5C regulates the incidence of severe preeclampsia by adjusting the expression of bone morphogenetic protein-7. *Bioengineered.* Apr;13(4):8538-8547. doi: 10.1080/21655979.2022.2051840.

Sourvinou IS, Markou A, Lianidou ES. 2013. Quantification of Circulating miRNAs in Plasma: Effect of Preanalytical and Analytical Parameters on Their Isolation and Stability. *The Journal of Molecular Diagnostics* 5(6):827–34. https://www.sciencedirect.com/science/article/pii/S1525157813001372

Sun M, Na Q, Huang L, Song G, Jin F, Li Y, et al. 2018. YAP Is Decreased in Preeclampsia and Regulates Invasion and Apoptosis of HTR-8/SVneo. *Reproductive Sciences* 25(9):1382–93. https://doi.org/10.1177/1933719117746784

Timofeeva AV, Fedorov IS, Sukhova YV, Ivanets TY, Sukhikh GT. 2023. Prediction of Early- and Late-Onset Pre-Eclampsia in the Preclinical Stage via Placenta-Specific Extracellular miRNA Profiling. *Int J Mol Sci.* Apr 28;24(9):8006

Ura B, Feriotto G, Monasta L, Bilel S, Zweyer M, Celeghini C. 2014. Potential role of circulating microRNAs as early markers of preeclampsia. *Taiwan J Obstet Gynecol* 53(2):232–4. https://www.sciencedirect.com/science/article/pii/S1028455914000783

Varelas X. 2014. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. *Development.* Apr 15;141(8):1614–26. https://doi.org/10.1242/dev.102376

Xin, H., Wang, C., Chi, Y. et al. 2020. MicroRNA-196b-5p promotes malignant progression of colorectal cancer by targeting ING5. *Cancer Cell Int* **20**, 119. https://doi.org/10.1186/s12935-020-01200-3

Xu P, Ma Y, Wu H, Wang YL. 2021 Placenta-Derived MicroRNAs in the Pathophysiology of Human Pregnancy. *Front Cell Dev Biol* ;9. https://www.frontiersin.org/articles/10.3389/fcell.2021.646326

Yadava SM, Feng A, Parobchak N, Wang B, Rosen T. 2021. miR-15b-5p promotes expression of proinflammatory cytokines in human placenta by inhibiting Apelin signaling pathway.

*Placenta*.104:8–15. https://www.sciencedirect.com/science/article/pii/S0143400420304392

Yang M, Chen Y, Chen L, Wang K, Pan T, Liu X, et al. 2016. miR-15b-AGO2 play a critical role in HTR8/SVneo invasion and in a model of angiogenesis defects related to inflammation. *Placenta*. 2016;41:62–73. https://www.sciencedirect.com/science/article/pii/S0143400416300443

Zhong Y, Zhu F, Ding Y. 2019. Differential microRNA expression profile in the plasma of preeclampsia and normal pregnancies. *Exp Ther Med*. Jun 3;

Zhou F, Sun Y, Gao Q, Wang H. 2020. microRNA-21 regulates the proliferation of placental cells via FOXM1 in preeclampsia. *Exp Ther Med*. Jun 24