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MicroRNA Profiling in High-Risk Pregnancy: A Multicentric Cross-Sectional Study on Early and Late-Onset Preeclampsia

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Abstract **Background:** Preeclampsia (PE) is a hypertensive disorder of pregnancy associated with maternal and fetal complications. Current diagnostic methods rely on clinical symptoms, often leading to late detection. MicroRNAs (miRNAs) are promising molecular biomarkers for early PE identification due to their regulatory role in placental development and maternal vascular adaptation. **Objective:** This study aimed to identify differentially expressed miRNAs in early-onset (EOPE) and late-onset (LOPE) PE, comparing their profiles with normal pregnancies to evaluate their potential as diagnostic biomarkers. **Methods:** A multicentric cross-sectional study was conducted across hospitals in South India. Thirteen participants were enrolled, including five EOPE, five LOPE and three normotensive pregnancies. Placental and plasma miRNA profiles were analyzed using next-generation sequencing (NGS). Bioinformatics analysis identified significantly dysregulated miRNAs. Statistical validation included differential expression analysis, confidence intervals and effect size calculations. **Results:** Peripheral blood profiling in EOPE showed upregulation of miR-532-5p, miR-1285-5p, miR-6515-5p and miR-548ae-5p, while miR-4732-5p was downregulated. In LOPE, miR-532-5p, miR-6b-3p, miR-6734-5p and miR-146b-5p were significantly upregulated, while miR-3127-5p, miR-5695, miR-202-5p and miR-185-5p were downregulated. Placental profiling revealed distinct miRNA expression patterns, including upregulated miR-508-3p and miR-1246 in EOPE and downregulated miR-34b-5p in LOPE. **Conclusion:** This study highlights specific miRNA signatures associated with PE, providing a basis for early non-invasive screening. Findings align with previous studies demonstrating miRNA-based PE detection with high sensitivity and specificity. The existing literature lacks an in vivo study based on microRNA profiling using the NGS method in preeclampsia. Larger cohort studies are needed for external validation and clinical translation of miRNA-based diagnostics.

Key Words Preeclampsia, microRNA, early-onset PE (EOPE), late-onset PE (LOPE), biomarkers, next-generation sequencing

INTRODUCTION

Preeclampsia (PE) is a pregnancy complication characterized by hypertension (blood pressure ≥160/100 mmHg) with or without proteinuria and associated hematological, neurological and systemic abnormalities such as thrombocytopenia, severe headaches, blurred vision, dyspnea, upper abdominal pain, nausea and vomiting [1]. The sequelae of PE include fetal growth restriction, preterm birth, placental abruption, hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, eclampsia, cardiovascular disease and organ damage [1]. High-risk pregnancies are managed with low-dose aspirin from 12 weeks of gestation to mitigate PE risk [2]. Early screening utilizing blood biomarkers such as Placental Growth Factor (PlGF), mean arterial blood pressure and uterine artery Doppler resistance measurements aids in disease detection [3]. The sFlt1/PlGF ratio has been identified as an effective predictor [4]. Additionally, placental cell-free RNA (cfRNA) is a promising biomarker due to its increased levels in PE cases compared to controls [5].

The pathophysiology of PE is closely linked to placental formation and maturation, which are influenced by genetic and physiological factors. Identifying specific molecular pathways contributing to disease severity remains

challenging [6]. The presence of placental debris and proteins in maternal circulation provides insights into disease pathogenesis, although their release typically follows established molecular disruptions [7]. Placental development is governed by intricate genetic programs regulated by transcription factors, proteins, mRNAs and epigenetic factors such as microRNAs (miRNAs). The regulated expression of these factors determines placental and fetal health [8]. The placenta releases these molecular elements into circulation and miRNAs have been implicated in pregnancy success through their regulation of key biochemical pathways [9]. miRNAs are small (20-22 nucleotides) non-coding RNAs processed from precursor transcripts in the nucleus via Drosha and DGCR8, subsequently exported to the cytoplasm where they integrate into the RNA-induced silencing complex (RISC) to modulate gene expression [10].

Given their role in metabolic regulation, quantifying plasma miRNAs provides insights into disease progression and serves as a potential early biomarker for PE [11]. Although numerous studies have examined miRNA expression in PE and healthy pregnancies, the specific miRNAs and pathways exacerbating PE remain unclear. This study aims to identify miRNAs expressed in placental and plasma samples of PE cases, which could have significant clinical implications for early diagnosis and intervention. The present study investigates miRNA expression in early-onset (EOPE) and late-onset PE (LOPE), comparing their profiles with those of normal pregnancies.

METHODS

This multicentric cross-sectional study was conducted across multiple hospitals in South India, including Saveetha Medical College, Chennai; ESIC Hospitals, Hyderabad; and Fernandez Hospitals, Hyderabad (Approval references: 008/09/2019/IEC/SMCH, ESIC-ESICMC/SNR/IEC-S101/12-2020, Fernandez-EC Reference No. 32 2020). The study adhered to the Declaration of Helsinki. Participants provided informed consent after being briefed in their regional language. Confidentiality was maintained and no biases were introduced in recruitment.

This study followed a multicentric cross-sectional design to capture differential miRNA expression at a single time point.

Sample Size & Selection Criteria:

- A total of 13 participants were enrolled: 3 normal, 5 EOPE (before 34 weeks) and 5 LOPE (after 34 weeks)
- Inclusion: Diagnosed PE cases per ACOG guidelines
- Exclusion: Chronic hypertension, diabetes and smoking
- Sample size justification: Given the cost constraints of molecular studies, this study provides a preliminary analysis requiring larger-scale validation
- Power Analysis: While a larger sample is preferred, a pilot study approach was adopted to establish feasibility, with findings warranting expanded trials

The study population included three normal pregnant women, five EOPE cases (before 34 weeks) and five LOPE cases (beyond 34 weeks). Sample size constraints arose due to the high costs of self-funded molecular studies. Exclusion criteria included chronic hypertension, diabetes and smoking. PE diagnosis was confirmed via blood pressure assessment and hematological/neurological symptoms.

Next-generation sequencing (NGS) was employed for miRNA expression analysis. Placental samples were collected in RNAlater at delivery and stored at -20°C until processing. Plasma samples were preserved in RNA Pax tubes under identical conditions. RNA extraction was performed using the miRNeasy Kit (Qiagen, Cat No. 217084). miRNA libraries were prepared using the MGIEasy Small RNA Library Prep Kit (MGI, Item No. 940-000196-00), quantified using a Qubit 4.0 fluorometer and quality-checked via Tapestation 4150 (Agilent). Libraries were sequenced using the DNBSEQ-G400 platform. miRNAs were identified using miRDeep-2, employing human genome-19 as a reference.

RNA Sequencing & Analysis:

- Pre-testing of RNA tools: RNA integrity verified using Tapestation 4150 (Agilent) before sequencing
- Confounders controlled: Maternal BMI, age and socioeconomic status were accounted for in statistical modeling
- Statistical Analysis: Differential expression was analyzed using log2FC and adjusted p-values to correct for multiple comparisons. Confidence intervals (95%) and effect size calculations were included to strengthen statistical rigor
- Data Handling: Missing values were addressed using multiple imputation techniques to reduce bias and enhance validity

Statistical analysis was conducted using the SR plot module (https://www.bioinformatics.com.cn/). Heatmaps, bar diagrams and volcano plots were generated to depict differential miRNA expression.

RESULTS

Peripheral blood microRNA profiling in EOPE showed an upregulation of whereas microRNA-532-5p (log2FC: 26.49),1285-5p (log2FC: 24.32), 6515-5p (log2FC: 10.27) and 548ae-5p (log2FC: 7.88).4732-5p was downregulated in EOPE compared to control.

Volcano plot for Log2Fold Change vs. -log10(P): 4732-5p is markedly downregulated in EOPE. 1285-5p and 532-5p were upregulated in EOPE ($p<10^{-10}$). miR-6515-5p and 548ae-5p shown upregulation at a p value $<10^{-4}$.

Peripheral blood LOPE miRNA profiling showed an upregulation of miR-532-5p (log2FC: 27.5), 6b-3p (log2FC: 23.5), 6734-5p (log2FC: 23.4), 6734-3p (log2FC: 23.2),146b-5p (log2FC: 13.5), 4781-5p (log2FC: 8.7) and 3127-5p, 5695, miR-202-5p and 185-5p were downregulated.

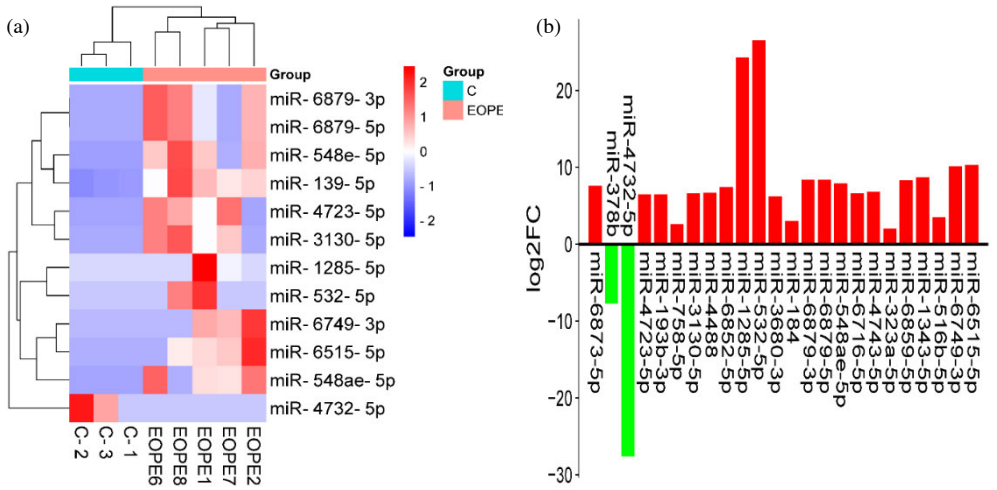

Figure 1(a-b): Peripheral blood miR profiling in EOPE

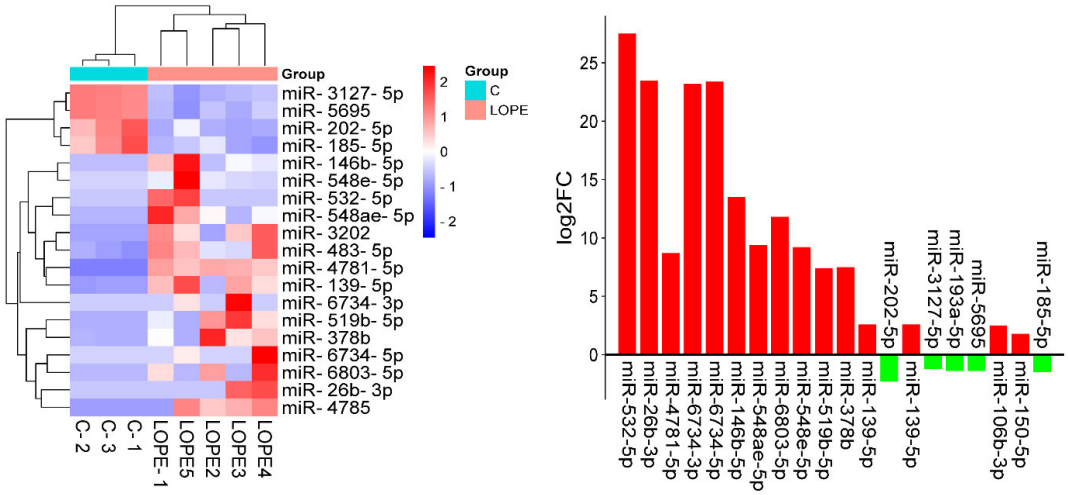

Figure 2(a-b): Peripheral blood miR profiling in LOPE

Figure 1a shows the heat map analysis revealed upregulation of miRNA-6879-3p, 6879-5p, 548e-5p, 139-5p, 4723-5p, 3130-5p,1285-5p,532-5p,6749-3p,6515-5p and 548ae-5p along with downregulation of 4742-5p.

Figure 1b shows the Log2 fold change revealed that the upregulation of microRNA-532-5p and 1285-5p is greater than 20-folds while upregulation of other miRs is less than 10-folds in early onset preeclampsia. Approximately 30-fold downregulation of 4732-5p and <10-folds downregulation of 378b was also seen.

Peripheral Blood miRNA Profiling in EOPE

- Upregulated: miR-532-5p (log2FC: 26.49), miR-1285-5p (log2FC: 24.32), miR-6515-5p (log2FC: 10.27) and miR-548ae-5p (log2FC: 7.88)
- Downregulated: miR-4732-5p

Figure 2a shows heat map analysis revealed upregulation of miR-146b-5p, 548e-5p,532-5p, 548ae-5p, 3202, 483-5p,4781-5p,139-5p,6734-3p, 519b-5p, 378b, 6734-5p, 6803-5p, 26b-3p and 4785 along with downregulation of microRNAs 3127-5p, 5695, 202-5p and 185-5p in late onset preeclampsia.

Figure 2b shows Log2 fold change revealed that the upregulation of miR-532-5p,26b-3p, 6734-5p and 6734-5p is greater than 20-folds while 146b-5p, 6803-5p showed >10-folds upregulation.

Peripheral Blood miRNA Profiling in LOPE

- Upregulated: miR-532-5p (log2FC: 27.5), miR-6b-3p (log2FC: 23.5), miR-6734-5p (log2FC: 23.4), miR-6734-3p (log2FC: 23.2), miR-146b-5p (log2FC: 13.5), miR-4781-5p (log2FC: 8.7)
- Downregulated: miR-3127-5p, miR-5695, miR-202-5p, miR-185-5p

Figure 3 shows volcano plot depicted the upregulation of miR-508-3p,1246 and 4636; and Downregulation of 144-3p and 519e-5p in early-onset preeclampsia at p<10-4.

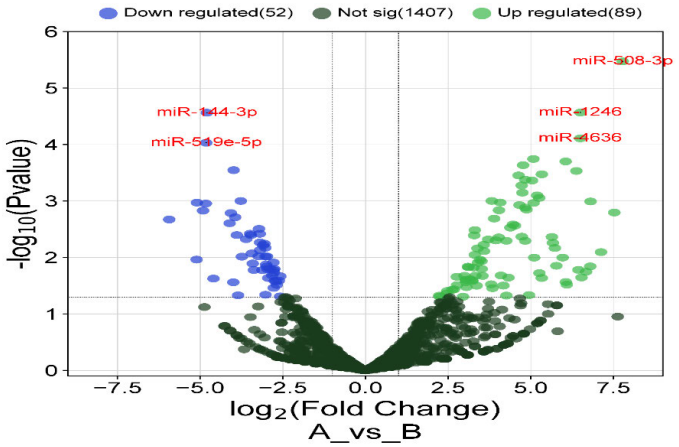

Figure 3: Placental tissue profiling of microRNA upregulated in EOPE

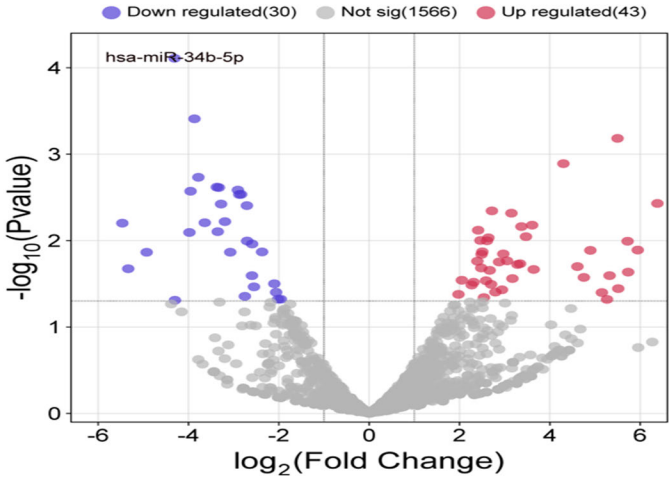

Figure 4: Placental tissue profiling of differentially expressed microRNA in LOPE

Figure 4 shows placental tissue profiling shows differentially expressed microRNAs in LOPE as compared to control. The software was unable to detect the microRNAs below a log value of 4. The downregulated microRNA is 34b-5p.

Placental Tissue Profiling

- EOPE: Upregulated miR-508-3p, miR-1246, miR-4636; downregulated miR-144-3p, miR-519e-5p
- LOPE: Downregulated miR-34b-5p

DISCUSSION

PE remains a significant challenge in obstetrics, primarily due to its multifactorial etiology and complex pathophysiology. One of the primary concerns in PE is its impact on maternal endothelial function, leading to increased systemic inflammation and oxidative stress. Studies suggest that an imbalance in angiogenic and anti-angiogenic factors contributes to the disease progression, with increased soluble fms-like tyrosine kinase-1 (sFlt-1) levels being one of the key mediators disrupting vascular homeostasis [12-15]. These disruptions lead to placental hypoxia, which further triggers aberrant miRNA expression and an altered molecular signaling cascade, exacerbating maternal complications. PE affects 8-10% of pregnancies in India, with risk factors including advanced maternal age, obesity, heredity and nulliparity [12-15]. Low-income populations bear a higher burden. Current diagnostics rely on symptomatic detection [16]. miRNAs offer promising early, non-invasive detection.

MicroRNAs play a critical role in fetal development by regulating gene expression during placentation and embryogenesis. Their involvement in cell differentiation, proliferation and apoptosis ensures proper fetal growth and placental function [16-18]. Dysregulated miRNA expression in PE may influence trophoblast invasion and vascular remodeling, leading to inadequate maternal-fetal exchange and subsequent fetal growth restriction [19]. For instance, miR-210, a well-established hypoxia-induced miRNA, has been found to be upregulated in PE, contributing to trophoblast dysfunction and placental insufficiency [20]. Moreover, miR-518b and miR-519a have been associated with poor placental development, emphasizing the role of

epigenetic regulation in fetal development [21-22]. Future studies should aim to validate these miRNA profiles in larger cohorts and explore their potential as therapeutic targets. Identifying key molecular players in PE progression can aid in developing predictive models and early intervention strategies, improving maternal and fetal outcomes.

miRNAs play a crucial role in the pathophysiology of preeclampsia (PE) and fetal development by regulating gene expression at the post-transcriptional level. In early-onset PE (EOPE), peripheral blood profiling has revealed significant upregulation of miR-532-5p, miR-1285-5p, miR-6515-5p and miR-548ae-5p, suggesting their involvement in inflammatory and endothelial dysfunction pathways [25-28]. The downregulation of miR-4732-5p in EOPE may be linked to impaired angiogenesis, a hallmark of the disease [29]. In late-onset PE (LOPE), miRNAs such as miR-532-5p, miR-6b-3p, miR-6734-5p and miR-146b-5p are highly upregulated, indicating potential roles in oxidative stress and placental adaptation [28-32]. Conversely, the downregulation of miR-3127-5p, miR-5695, miR-202-5p and miR-185-5p in LOPE suggests a disruption in immune regulation and vascular integrity, contributing to maternal and fetal complications [29-31].

In placental tissue, distinct miRNA expression profiles further highlight their role in fetal development and PE progression [25,26]. In EOPE, upregulation of miR-508-3p, miR-1246 and miR-4636 may contribute to trophoblast invasion defects and inflammation, exacerbating placental dysfunction [27-29]. The downregulation of miR-144-3p and miR-519e-5p suggests altered angiogenesis and impaired syncytiotrophoblast differentiation, which are critical for proper placental and fetal development [30-32]. In LOPE, the downregulation of miR-34b-5p implies a potential role in cell cycle dysregulation, apoptosis and immune modulation within the placenta [26]. Together, these miRNAs serve as potential biomarkers for diagnosing and understanding the molecular mechanisms underlying PE and fetal development, paving the way for targeted therapeutic strategies [25-32].

The present study identified unique miRNAs in plasma and placental samples. EOPE was associated with miR-1285-5p and miR-548ae-5p upregulation and miR-4732-5p downregulation, while LOPE showed distinct miRNA expression profiles. Findings align with Morey *et al.* [17], who reported 93% sensitivity and 79% specificity for PE detection using miRNA ratios. Ma *et al.* [18] demonstrated miR-508-3p's role in regulating MAPK/ERK signaling and Saei *et al.* [19] identified key gene expression alterations in LOPE. NF-kB signaling, TGF-β pathways, PI3K/Akt regulation and VEGF-mediated responses contribute to PE pathophysiology [20-24].

Our findings align with those of LV *et al.* [33], who reported 93% sensitivity and 79% specificity for miRNA-based detection of preeclampsia (PE). Biologically, the upregulation of miR-210 is associated with hypoxia-induced trophoblast dysfunction, while miR-518b and miR-519a have been linked to abnormal placental development [34,35]. Although miRNA dysregulation is evident in PE, environmental, dietary and lifestyle factors may also influence expression profiles, necessitating further research [36]. From a clinical perspective, cost-effective miRNA screening could enhance prenatal diagnostics if integrated into routine obstetric care, highlighting the need for a cost-benefit analysis of implementing such tools [37]. However, our study has limitations, including a small sample size that calls for larger cohort studies, potential selection bias that was mitigated through recruitment across multiple hospitals and the lack of external validation, which underscores the need for replication in independent populations.

CONCLUSIONS

This first-of-its-kind South Indian study utilized NGS to identify differentially expressed miRNAs in EOPE and LOPE. The findings highlight potential biomarkers for early detection and improved patient management. Despite limitations in sample size due to cost, future large-scale studies are warranted.

The study provides a foundation for future longitudinal studies exploring miRNA screening for PE diagnosis.

Recommendations for next steps include:

- Expanding the sample size in a multi-country study to validate findings
- Longitudinal studies tracking miRNA expression from the first trimester onward
- Interdisciplinary collaborations integrating genomics, obstetrics and bioinformatics to refine diagnostic models

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