Review article

Comparative Analysis of MicroRNAs and Proteinuria Testing for Early Diagnosis of Pre-eclampsia

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Pre-eclampsia (PE) is a life-threatening condition affecting pregnant females worldwide. PE poses significant risks to maternal and fetal health, necessitating early detection for timely management. Traditional diagnostic methods like proteinuria testing face limitations in sensitivity and specificity. Proteinuria can occur in other medical conditions other than PE. To address the challenge in PE detection, microRNAs (miRNAs) are emerging as biomarkers for early diagnosis. MiRNAs are small non-coding molecules that regulate gene expression. MiRNAs play important roles in PE pathogenesis, with dysregulated expression implicated in the disease. The release of miRNAs into circulation offers non-invasive detection options. While challenges like variability in expression profiles exist, miRNAs offer higher sensitivity and specificity compared to proteinuria testing. Standardized protocols and miRNA biomarker panels are needed for routine clinical use. Integrating miRNA analysis into existing diagnostic protocols holds promise for improving the accuracy of PE diagnosis and facilitating timely intervention to improve maternal and fetal outcomes.

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Introduction

Pre-eclampsia (PE) is a complex pregnancy complication affecting 2-8% of expectant females worldwide.[1-3] Various maternal risk factors, including previous medical history, advanced age, and the presence of comorbidities, increase the likelihood of developing PE. The features of this condition include hypertension, protein in urine (proteinuria), generalized edema, fetal growth restriction (FGR), and damage to organs like the kidneys and liver.[1,3-5] Beyond increased pregnancy risks, patients with PE are at an increased long-term risk of cardiovascular disease (CVD), hypertension, and type 2 diabetes later in life.[1,6-7] This condition contributes not only to the maternal disease burden but also to fetal morbidity and mortality due to FGR, placental abruption, or preterm deliveries.

Despite the severe consequences, the early detection of PE is still a challenge because of the complex etiology and pathophysiology, leading to missed opportunities for timely intervention and management.[9,10] Traditionally, assessing maternal risk factors, blood pressure monitoring for pregnant females and testing for protein in urine are used for PE diagnosis. Suspected cases of PE require a thorough evaluation of the patient's medical history. However, even with a full medical history the detection rates based on maternal risk factors remain low at less than five percent.[10,11] Moreover, proteinuria testing traditionally used to identify potential PE varies in sensitivity and specificity among patient's. Other conditions like chronic kidney disease (CKD) can complicate the diagnosis of PE due to elevated protein in urine, highlighting the need for more reliable diagnostic approaches.

To address these challenges, recent advancements in biomarker applications provide new avenues for the early prediction of PE. The ratio of biomarkers such as placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) is used as a predictive test for early PE diagnosis.[1,4-5,7,9] However, the sFlt-1:PlGF ratio has a low positive predictive value, low sensitivity, and a high negative predictive value, posing a challenge in PE detection.[7] Consequently, failure to detect PE can have negative consequences to the mother and fetus.

The use of reliable biomarkers for PE detection have the potential to improve patient outcomes. Other biomarkers like microribonucleic acids (miRNAs), are promising candidates for early PE diagnosis.[11,12] MiRNAs are sensitive and accurate biomarkers in comparison to proteinuria testing for the early diagnosis of pre-eclampsia.

Background

PE is a hypertensive disorder in pregnancy of significant concern in maternal and fetal health due to the potential for serious complications for the mother and the unborn child. PE is characterized by significant proteinuria and high blood pressure of 140/90 mmHg, obtained on at least two occasions measured four hours apart, in previously normotensive females, beginning mostly in the second trimester.[1,4,8,13] The high blood pressure reading may or may not be accompanied with symptoms like edema of the face, hands, and legs, with protein in urine. Also, the signs of maternal organ dysfunction such as impaired liver function, renal insufficiency, pulmonary edema, or neurological symptoms may accompany PE. The complications of PE include eclampsia (seizures), HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), placental abruption, FGR, stillbirth, and preterm birth.[1,4,13-14] Moreover, preterm infants are at an increased risk of developmental delays like cerebral palsy.[4,15] These outcomes, in addition to the long-term risk of mothers developing other disease conditions, increase the burden of maternal and infant morbidity and mortality worldwide.

Annually, PE contributes to 76,000 maternal and 500,000 infant deaths worldwide, with higher rates in low-income countries.[1,7] Females in developing countries are at a 14 times higher risk of dying from obstetric complications compared to those in developed

countries.[14] Scarce resources and lack of access to early diagnosis and medical care contribute to the higher mortality rates[7,16] PE alone is responsible for approximately 12% of maternal deaths globally.[14] Maternal hypertensive disease accounted for 6.6% of pregnancy-associated deaths in the United States between 2014 and 2017, with the rates at 26% in Latin America and the Caribbean, and 9% in Africa and Asia.[1,2]

Despite the severity and global impact, the exact cause of PE is not fully understood. The etiology of PE is believed to involve a complex combination of factors like genetics, immune system dysfunction, and abnormal placental development.[1,7,17] Genetic predisposition is a significant factor as evidenced by identifycation of genetic variations and familial clustering analyzed from hospital records, showing increased risk of PE in the sisters, daughters, and granddaughters of women with PE compared to their sisters-in-law.[18] In addition, the genetic variations may affect biological pathways involved in vascular function, endothelial function, and immune regulation.[18]

The dysfunction of the endothelium, the cells lining the blood vessels, affects various organs throughout the body and is characterized by increased vasoconstriction, increased vascular permeability, and activation of the coagulation cascade.[8,19] In the kidneys, impaired endothelial function reduces glomerular filtration rate and causes proteins normally retained in the blood, such as albumin, to leak into urine, due to increased permeability of the blood vessels.[8] As a result, renal dysfunction, and water retention, which manifests as generalized edema, occurs. Similarly, in the liver, it can cause damage to the hepatic sinusoidal endothelium, resulting in liver dysfunction and abnormalities in blood clotting mechanisms. Brain-related consequences of endothelial dysfunction include cerebral edema and manifestations of neurological symptoms like headaches, visual disturbances, and seizures.

Endothelial dysfunction also results from the dysregulation of the maternal immune system, implicated in the etiology of PE, showing the complex association between maternal physiology and pregnancy outcomes. In a normal pregnancy, the maternal immune system undergoes changes to accommodate the developing fetus. The changes involve a complex interaction between maternal immune cells and fetal cells, which are foreign to the mother, to establish immunological tolerance and prevent the rejection of the fetus.[20] In PE, however, immune responses may occur, leading to production of immune factors, like pro-inflammatory cytokines, which lead to systemic inflammation and dysfunction of the endothelium.[21] The immune abnormalities increase the risk of PE complications such as HELLP syndrome and placental abruption.

PE complications also arise from abnormal placental development and function, another factor involved in the pathogenesis of the condition. During the early stages of pregnancy, the trophoblast cells, the outer layer of cells of the embryo that later develop into a placenta, invade the uterine spiral arteries (SA), and form new blood vessels, a process called angiogenesis.[6,22] The SA are transformed into wider, low-resistance vessels, facilitating increased blood flow to the placenta for fetal-maternal exchange of waste and oxygen.[21] However, in PE, this invasion is shallower, leading to poor placental implantation and narrow SA, which results in inadequate placental perfusion and hypoxia, resulting in oxidative stress.[6] Impaired placental perfusion deprives the fetus of oxygen and nutrients, leading to FGR.

Additionally, the hypoxic placenta triggers the release of anti-angiogenic factors like sFlt-1 into maternal circulation in higher amounts compared to PlGF, an angiogenic factor. The imbalance of the factors leads to the reduced formation of blood vessels and increased vascular permeability, which in turn exacerbates endothelial dysfunction and systemic inflammation.[4,7,17,19,22] This cascade of events is what leads to the clinical manifestation of PE such as FGR, hypertension, edema, and proteinuria along with complications like

placental abruption, preterm birth, eclampsia, and HELLP syndrome.[19]

Despite the research on PE implications, there is no effective definitive treatment. Delivery of the placenta is the primary intervention to reduce adverse outcomes.[6,8-9] However, the fetal survival rates are lower in early pregnancy, requiring consideration of the timing of delivery and the overall health of mother and the infant. Furthermore, preterm infants may need intensive care and are at an increased risk of developing conditions like cerebral palsy, diabetes, and hypertension later in life.[9,15] Infants from mothers who had PE are at a 2.5 times higher risk of developing hypertension than those born from normotensive pregnancies.[9] Emerging evidence suggests that low doses of aspirin coupled with monitoring of the mother and fetus show promise in reducing disease progression in the early gestational stages by 62%, subsequently avoiding adverse outcomes.[8,15]

Identifying females at risk of developing PE can mitigate the negative outcomes through early intervention. High-risk factors for PE, such as a history of PE, renal or autoimmune diseases, diabetes, and chronic hypertension are associated with systemic inflammation, endothelial dysfunction, and vascular abnormalities, seen in PE pathogenesis, heightening the risk.[4,8] A history of PE increases the risk eight times, due to existing vascular dysfunction, making it the highest risk-factor.[4,7] Females with moderate-risk factors, such as obesity, older age, and a family history of PE are at a higher risk due to existing endothelial dysfunction. The risk in nulliparous females, who have never given birth before, is associated with the lack of immunological adaptations that occur during subsequent pregnancies, increasing susceptibility to placental dysfunction and immune-mediated responses.[4,7,23] Sociodemographic factors like low socioeconomic status and limited access to healthcare may exacerbate the PE risks, due to inadequate prenatal care.[8,16] This evidence indicates a critical need for the thorough assessment of risk factors and medical history in suspected cases for early intervention.

Assessing maternal risk factors and periodic measurement of blood pressure and urine protein levels during prenatal visits are conventional methods used to diagnose PE.[1,7,14] However, these methods lack sensitivity and specificity for predicting PE, especially in nulliparous females or those without a history of PE.[1-2] Development of more sensitive diagnostic methods will ensure the early detection and diagnosis of PE.

To develop effective diagnostic methods, emerging biomarker research explores the use of factors released into maternal circulation in PE, focusing on impaired angiogenesis and the dysregulation of miRNAs.[17,22,24] Measuring the levels of anti-angiogenic factors like sFlt-1 in a ratio to the angiogenic factor PlGF, demonstrates promise in excluding females at increased risk of developing PE, due to the high negative predictive value.[1,3,5,17,22] However, the ratio has a low sensitivity and a low positive predictive value, which may lead to missing positive PE cases.[7,9]

Recognizing the limitations, efforts have been made to enhance predictive accuracy through innovative approaches, such as using the first-trimester screening algorithm. The algorithm combines maternal demographic characteristics, medical history, biomarkers, and ultrasound, to improve the accuracy of PE prediction.[1-2] Additionally, research in miRNAs involvement in PE as potential biomarkers is showing promise for early diagnosis.[6,12,24]

MiRNAs, small non-coding RNA molecules that play important roles in gene regulation, are emerging biomarkers for PE prediction.[12,25] In pre-eclamptic pregnancies the miRNAs are dysregulated, affecting processes such as angiogenesis, placental development, and immune response.[6,19,26] Measurement of miRNA levels in maternal circulation may serve as a sensitive and specific biomarker for early PE diagnosis, providing an opportunity for timely intervention and management.

Proteinuria Testing

Proteinuria testing is a diagnostic procedure used to detect abnormal levels of protein in urine, which can indicate various underlying health conditions including renal diseases, CVD, and PE. Assessing abnormal levels of protein in urine is a standard diagnostic criterion that complements blood pressure monitoring and the assessed maternal risk factors in PE diagnosis. Proteinuria testing is recommended for pregnant females with two blood pressure readings of more than 140/90 mmHg, to diagnose PE.[4]

To perform proteinuria testing, a sample of urine is required, and must be collected in a sterile, leak-proof container. Patients are advised to follow proper hygiene practices, like handwashing, to minimize contamination of the urine sample. Additionally, care should be taken to avoid introducing toilet paper, stool, or menstrual blood into the sample, which can interfere with test results.[27] Depending on the diagnostic method available, random, and 24-hour urine samples are used for proteinuria testing.

Proteinuria can be detected through dipstick testing, urine protein-to-creatinine ratio (UPCR) assessment, and 24-hour urine analysis methods. Dipstick testing provides a qualitative assessment, where a chemical strip is immersed in a urine sample and a color change indicates the presence or absence of protein. The intensity of color change on the dipstick corresponds to the amount of protein present in the sample. If there is a color change, a confirmatory quantitative test, such as UPCR analysis, where protein and creatinine levels in a urine sample are measured and the ratio calculated to determine the degree of proteinuria, is performed. Alternatively, a 24-hour urine protein quantification, where all urine excreted over a 24-hour period is collected and analyzed for total protein content is used. The threshold for PE diagnosis is a protein concentration of ≥30 mg/mmol UPCR or ≥300 mg in a 24-hour urine sample.[4,13,27] In areas where confirmatory tests are not available, a dipstick test reading of ≥2+ is considered significant in the diagnosis of PE.

Proteinuria testing has challenges and is susceptible to limitations associated with the pre-analytical, analytical, and post-analytical phases that can lead to missed or incorrect diagnoses of PE. Pre-analytical limitations, such as improper sample collection, inadequate sample storage, or transportation conditions, can impact the integrity of the urine sample and subsequent test results. For example, collecting a 24-hour urine sample, while considered the gold standard for proteinuria detection, is time-consuming and inconvenient for patients.[7,27] Moreover, contamination during sample collection may result in unreliable test results, affecting the accuracy of PE diagnosis. Analytical limitations, consisting of issues with the testing process, such as assay variability, cross-reactivity with other substances present in urine, or interference from medications, can lead to inconsistencies in proteinuria measurement. Post-analytical limitations encompassing challenges related to result interpretation, reporting errors, or data transmission issues, lead to misinterpretation or miscommunication of test findings, potentially affecting clinical decision-making regarding PE diagnosis and management.

Adding to the limitations, proteinuria testing lacks sensitivity and specificity and does not occur in all cases of PE. False positive results can occur in conditions other than PE, such as chronic hypertension, CKD, or urinary tract infections (UTI), which are characterized by proteinuria.[28] Distinguishing PE-related proteinuria from proteinuria caused by other underlying conditions can be challenging, leading to diagnostic confusion. False negative results, on the other hand, can lead to missed diagnoses. Approximately 10% of cases do not manifest with detectable proteinuria levels and 43% of severe PE cases demonstrate proteinuria levels below the standard threshold.[29] This variability in proteinuria questions the necessity of the clinical utility of the test,

particularly in severe cases of PE, where organ damage is already evident.

To address these challenges, guidelines are necessary to provide a standardized diagnostic protocol for diagnosing PE. According to guidelines issued by the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom and the American College of Obstetricians and Gynecologists (ACOG) in the United States (US), proteinuria is no longer mandatory for PE diagnosis, as some cases manifest without protein in urine.[4,8] The guidelines provide a comprehensive assessment of PE, considering a broader range of clinical features beyond proteinuria, like organ damage, and assessment of maternal risk factors to enhance PE prediction.

Despite the focus on improving the diagnostic assessment for PE, challenges persist in the efficacy of the screening guidelines including different detection rates and false positive rates. A comparison of the methods for PE detection showed that the ACOG guidelines have a higher detection rate of above 89% at the expense of a higher false-positive rate (FPR) at 64.2%, while the NICE guidelines have a lower detection rate of less than 41% with a lower FPR at 10.2%.[23] To mitigate the potential for missed diagnoses, more comprehensive and sensitive diagnostic approaches are necessary to facilitate the early detection of PE.

MiRNAs

Exploring innovative diagnostic tools, such as molecular biomarkers, can enhance the accuracy and timeliness of PE detection. An effective predictive test will contribute to early identification of PE and subsequently improve maternal and fetal outcomes.[30] MiRNAs have been suggested as potential biomarkers for the early diagnosis of PE.[3,6,12]

MiRNAs are small non-coding ribonucleic acid (RNA) molecules, containing about 18-22 nucleotides, which regulate more than 60% of the coding genes in humans.[19,25,31] The sequences encoding miRNAs are found in introns, the non-coding portion of the genomic deoxyribonucleic acid (DNA) that does not translate protein.[32-33] The synthesis of miRNAs starts in the nucleus, where DNA is transcribed into primary miRNA transcripts (pri-miRNAs), which are long precursor molecules containing a hairpin structure. The pri-miRNAs are cleaved at the base of the hairpin structure by a ribonuclease enzyme called Drosha into precursor miRNAs (pre-miRNAs), which are then exported to the cytoplasm by Exportin-5.[24]

In the cytoplasm, the pre-miRNAs are further cleaved at the loop of the hairpin structure by another enzyme called Dicer, generating a short RNA duplex consisting of the mature miRNA strand and a complementary sequence. The mature miRNA strand is then loaded into the RNA-induced silencing complex (RISC), a multiprotein complex that contains Argonaute (Ago) protein, which makes the miRNA stable.[19] The mature miRNA strand guides RISC to recognize and bind to target messenger RNA (mRNAs) through base pairing interactions.

The binding of the miRNA to the target mRNA results in gene regulation through post-transcriptional mechanisms. The binding can stop translation, cause mRNA degradation, or both, depending on the degree of complementarity between the miRNA and the target. Consequently, gene silencing occurs, leading to a decrease or absence of protein production.[19,34] This regulation of genes plays an important role in controlling gene expression and physiological cellular processes such as cell growth, cell differentiation, metabolism, apoptosis, regeneration and even pregnancy.[6,19] The dysregulation of miRNAs is implicated in the development of various diseases like cancer, CVD, metabolic diseases, and PE, due to altered physiological processes or gene expression.

The regulatory role of miRNAs is complex. A single miRNA can regulate several genes and one gene can be regulated by any number of miRNAs. The phenomenon of one-to-many regulation reflects the capacity of miRNAs to recognize and bind to complementary sequences of multiple mRNA targets.[19] A single gene, on the other hand, can have multiple binding sites for different miRNAs, allowing integration

of different signaling pathways and regulatory inputs by multiple miRNAs. The complex combinations of miRNA actions and the target genes contribute to the precise gene expression patterns and regulation of cellular processes.

MiRNAs are also versatile in their expression patterns, contributing to the specificity and functionality of various tissues and cell types. While some miRNAs exhibit tissue-specific or cell-type specific expressions, others can be present in all cell types, enhancing the function of the various tissue or cell processes. Placenta-specific miRNA, mi-155, for example, regulates gene expression patterns associated with placental development and function and has been identified in maternal circulation.[6,24,35]

Cells or tissues can release miRNAs into circulation in body fluids including blood, urine, and saliva in free form or bound to RNA-binding proteins or lipoproteins. Mature miRNAs are released into circulation directly by living cells, passively from apoptotic cells, or through active secretion through extracellular vesicles like exosomes.[6,36] Exosomes are small membrane-bound vesicles derived from the endosomal compartment and released into the extracellular space by fusing with the plasma membrane and are involved in intercellular communication. For example, placental derived exosomes facilitate communication between maternal and fetal cells and also play a role in promoting maternal immune tolerance to the fetus by inhibiting maternal T cell signaling components.[6] The miRNAs in exosomes are encapsulated within lipid bilayer membranes to facilitate transportation through the circulation and protection from degradation.[36-38] The presence of miRNA in circulation presents a potential for use as molecular biomarkers for the diagnosis of diseases like PE.

MiRNAs for Pre-eclampsia Diagnosis

The plasma levels of miRNAs are higher in pregnant females than non-pregnant females and decrease after delivery.[6,39] The miRNAs in pregnancy originate from the placenta or other gestational tissues and are released into circulation, reflecting the ongoing changes in gene expression and cellular activities to support fetal development and maternal adaptation.[11] These miRNAs play important roles in processes such as embryo implantation, placental development, and immune modulation.

Regulation of gene expression profiles by miRNAs is essential for trophoblast invasion to ensure successful embryo implantation to the uterine lining.[6] MiRNAs also influence placental development by facilitating trophoblast proliferation, differentiation, and angiogenesis, to ensure establishment of a functional placenta crucial for nutrient and oxygen exchange between mother and fetus. For instance, miR-376c promotes trophoblast proliferation and invasion, while miR-299 and miR-181a-5p inhibit these processes.[33] Additionally, miR-181a plays a role in modulating the maternal immune response to maintain maternal-fetal tolerance and still protecting the mother and fetus against pathogens. The specific functions of the miRNAs during pregnancy demonstrates the significance of the complex interactions of miRNAs.[33]

The complexity requires an understanding of the dysregulation of miRNAs. Dysregulation can disrupt the balance of pregnancy-related processes, leading to pregnancy complications such as FGR, PE and preterm birth.[6,25,33] Seven miRNAs, miR-125b, miR-518b, miR-628-3p, miR-365a-3p, miR-520h, miR-374a-5p, miR-191-5p, are significantly associated with a pregnancy complication.[25] Elevated levels of miR-125b and miR-518b miRNAs are directly associated with PE, while miR-374a-5p and miR-191-5p levels are increased in PE, preterm birth, and FGR, inhibiting trophoblast invasion and migration or causing endothelial dysfunction[6,25,33,40] Altered miRNA profiles of miR-210 and miR-155, are also associated with endothelial dysfunction and present in pre-eclamptic pregnancies compared to normal pregnancies.[24,33,35] The changes in the levels of miRNAs can potentially provide valuable information for monitoring pregnancy health and diagnosing pregnancy-related disorders.

MiR-210 is clearly associated with PE. The expression of miR-210 is induced under hypoxic conditions, which is commonly seen in the placenta of PE patients.[33,35] MiR-210 is elevated in PE and the dysregulation contributes to impaired trophoblast invasion, altered angiogenic balance, and increased oxidative stress.[22,33,35] The elevated expression levels in placenta, plasma, and sera of PE patients compared to controls make miR-210 a promising biomarker for PE diagnosis.

Other miRNAs with varying expression patterns in circulation or in the placenta occur in PE but require further studies. For, example, miR-200b-3p, measured using quantitative real-time polymerase chain reaction (qPCR), is significantly upregulated in PE placental tissues and contributes to trophoblast cell dysfunction by inhibiting proliferation and migration while promoting apoptosis.[41] MiR-141 is also enriched in the placenta and is downregulated in PE.[33] The differential expression of commonly identified miRNAs from various biological samples is shown in Table 1.

The methods to measure the differential expression of miRNAs are not standardized. Different methods demonstrate contrasting patterns on some miRNAs, such as miR-125b (Table 1). MiR-125b involved in angiogenesis and SA remodeling, is highly conserved in a species.[40] Studies indicate that miR-125b is upregulated in PE, then the levels decrease after delivery.[6,25,33,39] In contrast, another study by Gan and colleagues in 2017 report reduced expression levels of miR-125b in serum samples from women with PE compared to healthy controls but did not discuss the role in PE pathogenesis.[35]

Reduced miR-125b levels demonstrate initial overexpression in the first trimester from women who later developed PE and subsequent reduction as pregnancy progresses.[40] This links miR-125b and PE through the regulation of trophoblast cell surface antigen (Trop-2) protein expression in the syncytiotrophoblast and suggests miR-125b involvement in the early onset of PE. The biomolecular and morphological analyses indicate an underexpression of miR-125b and downregulation of Trop-2 in PE placenta in comparison to control samples.[40] The downregulation of miR-125b is linked to decreased expression of the target gene, Trop-2, demonstrating the complex miRNA and target gene interaction in placental function.

In addition to individual miRNAs, various miRNA clusters are abundantly expressed in the placenta. Clusters are groups of miRNA genes located close together that share similar functions or target genes.[33] The chromosome 19 miRNA cluster (C19MC) and chromosome 14 miRNA cluster (C14MC) in the placenta play roles in embryonic development and placental function.[6,33,42] The imbalance of miRNAs levels from these clusters causes placental dysfunction in PE leading to FGR.

The expression levels of miRNAs in the clusters vary with gestational age. MiRNAs from C19MC increase as the pregnancy progresses.[6,24] In contrast, miRNA genes from C14MC increase during the first trimester and reduce in the third trimester. The variation in miRNA levels can be leveraged as biomarkers to detect PE onset and potential complications, and subsequently facilitate early intervention.

Detection and quantitation of miRNA levels is performed using various molecular methods, including qPCR, microarray hybridization, and next generation sequencing (NGS). The precise quantification of miRNAs in a biological sample can be performed using qPCR. The amplifycation of a small sample combined with a microarray hybridization quantifies fluorescently labelled miRNAs by hybridization to complementary probes on an immobilized surface.[11,25] The degree of hybridization is detected with fluorescence signals quantified to determine the expression levels of the corresponding genes. On the other hand, NGS analyzes the comprehensive miRNA profile in a sample and does not use pre-designed probes.[43] NGS allows for the simultaneous analysis of many miRNAs in a single assay, offering insights into the complex regulatory

Table 1. Differential expression of miRNA and involvement in pre-eclampsia (PE)

Biological Source	MiRNA	Expression Level	Involvement in PE	References
Placenta, serum, urine	miR-210	Upregulated	Inhibits trophoblast invasion; associated with oxidative stress, mitochondrial dysfunction, and inhibits angiogenesis	[22,25,33,35]
Placenta, serum, urine	miR-155	Upregulated	Associated with inflammation, endothelial dysfunction, and apoptosis	[33]
Placenta	miR-376c	Downregulated	Promotion of trophoblast proliferation and invasion is suppressed	[33]
Placenta	miR-299	Upregulated	Inhibits trophoblast proliferation and invasion	[33]
Placenta, plasma	miR-181a-5p	Upregulated	Inhibits trophoblast proliferation and invasion; Contributes to immune imbalance at the maternal-fetal interface	[33]
Placenta	miR-200b-3p	Upregulated	Inhibits trophoblast cell proliferation and migration; promotes apoptosis	[33,41]
Plasma, serum	miR-125b*	Dysregulated	Inhibits trophoblast invasion, endothelial dysfunction	[25,33,35,39,40]
Plasma, serum, placenta	miR-518b	Upregulated	Impairs trophoblast migration and angiogenesis	[25,33]
Plasma	MiR-374a-5p	Upregulated	Down regulates pro-inflammatory markers; also associated with FGR and preterm birth	[25]
Plasma, placenta	miR-191-5p	Upregulated	Apoptosis, also associated with FGR and preterm birth; further study recommended	[25,33]

** Some studies identified overexpression while others downregulation*

networks involved in PE pathogenesis.[43-44] NGS is a more sensitive method compared to micro-array hybridization and qPCR.[43]

MiRNAs demonstrate a high sensitivity in detecting the early signs of PE. The sensitivity of circulating miRNAs for the diagnosis of PE is 0.88 (95% CI: 0.80–0.93), correctly identifying 88% of positive cases.[12] MiRNAs such as miR-210 and miR-155 show altered expression levels in the early stages of PE development, before the onset of hypertension and proteinuria, making them potential biomarkers for early PE diagnosis.[6,22,33,35] In addition, urine miR-210 and miR-155 have a positive correlation with 24-hour urine proteins, suitable for evaluating kidney damage in PE.[35]

In addition to a positive correlation with disease and high sensitivity, miRNAs also demonstrate high specificity. The specificity for circulating miRNAs in PE is 0.87 (95% CI: 0.78–0.92), indicating that 87% of patients without PE are identified correctly.[12] The sensitivity and specificity data indicates that the distinct expression of miRNA profiles in pre-eclamptic pregnancies compared to healthy controls, provides a potential for miRNA biomarkers use in diagnosis.

Despite the high sensitivity and specificity as potential biomarkers, challenges exist in detection and utilization of miRNAs in clinical applications. MiRNA expression profiles vary among individuals due to genetics, environmental, or physiological factors, making it difficult to have a universal diagnostic criterion.[26] Additionally, PE can manifest in different clinical presentations and severity levels, further complicating the identification of specific miRNA biomarkers to reliably distinguish affected and unaffected individuals across diverse populations.[29]

In addition to the physiological variation of miRNA levels, the complexity in interaction of

miRNAs that regulate multiple genes or PE regulation by multiple miRNAs poses a challenge. Processes such as angiogenesis, trophoblast invasion and endothelial function are regulated by various miRNAs with the dysregulation of the miRNAs implicated in PE pathogenesis.[6,19] No single miRNA can serve as a definitive biomarker for PE due to the complex pathogenesis and interconnectedness of miRNA-mediated regulatory networks. Instead, a panel that quantifies multiple miRNAs is necessary to represent the different variations in PE pathophysiology.

Besides the different variations, the miRNAs in clusters show sequence similarity presenting a challenge in correct detection, due to target ambiguity.[35] Techniques like qPCR and microarray hybridization lack the specificity to accurately differentiate between closely related miRNAs. qPCR relies on predesigned primers, while microarray hybridization uses probes, which may bind to unintended targets.[12] The non-specific binding potentially affects the accuracy of the results. The NGS method does not require hybridization and sequences individual miRNA, allowing for the detection of closely related sequences.[43]

Despite the advantage of NGS, the widespread implementation in clinical practice faces challenges. Firstly, NGS requires specialized equipment, which can be costly to buy and maintain. Secondly, NGS generates large amounts of sequencing data, requiring robust computational infrastructure and bioinformatics expertise.[45-46] Investing in computational resources, software tools, data storage solutions, and personnel training or outsourcing skilled personnel, adds to the costs.[45] The advantages and limitations of test methods for miRNA analysis must be carefully considered in clinical practice.

Discussion

The early diagnosis of PE is essential to reduce negative maternal and fetal outcomes. In addition to the risk of negative outcomes such as FGR or maternal organ failure during pregnancy, there is an increased risk for long-term development of CVD or diabetes in both the mother and the child later in life.[9] Maternal and fetal death can also occur if PE is not managed early. However, early detection is still challenging due to the complex etiology and pathophysiology and varied clinical presentation of PE.[8-9,29] MiRNA analysis is emerging as a superior detection method compared to proteinuria testing for the early diagnosis of PE.

Proteinuria testing has been used in the diagnosis of PE for decades. The method is simple, non-invasive, cost-effective, and widely available, and complements blood pressure monitoring in identifying patients at risk for PE. However, proteinuria testing has several limitations. Proteinuria can occur in other conditions such as chronic hypertension or renal diseases, leading to diagnostic confusion. False positives and false negatives are common, especially in cases where proteinuria is not present or is present at levels below the diagnostic threshold.[28-29]

Since the proteinuria symptom does not manifest in all cases of PE, the testing is no longer required for PE diagnosis, especially when other symptoms are present.[4,8] Nonetheless, the test is still valuable to assess maternal organ function. Proteinuria can provide insights to the extent of kidney damage and guide treatment decisions to mitigate complications.

To mitigate challenges with proteinuria testing, combining the test with maternal risk factors and organ failure assessment, ultrasound, and biomarker testing is a better predictor of PE than proteinuria testing alone. The first-trimester screening algorithms by ACOG and NICE guidelines for PE diagnosis are based on the combination of maternal demographic and medical history assessment, ultrasound measurements and biochemical markers.[4,8] The combined factors provide a more reliable method for predicting and diagnosing PE.

The reliability of the algorithms for PE diagnosis in low-resource settings is not well-studied. Resource-limited settings rely on proteinuria testing and assessment of maternal

clinical presentation, as biomarker assays are not routinely available.[8] More studies and the validation of the effectiveness of the protocols for early PE diagnosis, used in the settings, is needed.

In contrast to proteinuria testing, miRNA analysis has the potential to improve the early diagnosis of PE. MiRNAs play important roles in gene regulation, and in various biological processes relevant to PE pathogenesis, such as angiogenesis, placental development, and immune modulation.[11-12,35,41] The important function of miRNAs highlights the potential utility as biomarkers. Altered levels of miRNAs in PE can be detected earlier before proteinuria or hypertension manifests, offering an early diagnostic window.[6,33,35] Dysregulated miRNAs observed in pre-eclamptic pregnancies from various samples including placental tissues, blood, and urine, such as miR-210, have a high sensitivity and specificity for PE detection.[12,35] Furthermore, detection of miRNAs in maternal circulation offers a non-invasive approach, preferable for routine screening during prenatal care.

In addition to availability in circulation, miRNAs exist as stable complexes. MiRNAs form complexes with Ago protein or are encapsulated in extracellular vesicles. This increases their stability and prevents degradation by ribonucleases.[6,36,47] The stability of miRNAs offers an advantage for use as biomarkers.

Despite the potential for use as biomarkers, challenges exist in the practical implementation of miRNA-based diagnostics. Firstly, the variability in miRNA expression among individuals and the complexity of miRNA interactions necessitate the development of standardized diagnostic criteria. The inconsistent expression patterns of miR-125 in PE demonstrate the complexity of molecular mechanisms underlying the condition.[6,25,33,35,39-40] Developing and validating biomarker panels will address the multiple miRNA involvement in PE. Secondly, the testing methods, qPCR, microarrays, and NGS, are costly and not widely available.[43] Lastly, the clinical utility of miRNAs in PE diagnosis requires validation through large-scale prospective clinical studies to establish efficacy and reliability.[12,24,33,42]

However, amidst the challenges, miRNAs are a promising alternative to proteinuria testing in the early diagnosis of PE. While proteinuria testing offers valuable insights into the diagnosis of PE, miRNA analysis has a higher sensitivity and specificity. Integrating miRNA-based testing into existing diagnostic protocols can improve the accuracy of PE diagnosis and facilitate timely intervention. The standardization of procedures and validation of biomarker panels are necessary to ensure the reliability and accuracy of miRNA-based assays, to improve maternal and fetal outcomes.

Conclusion

PE poses a significant concern in maternal and fetal health globally. PE causes maternal organ failure, FGR and often results in severe complications such as preterm birth, stillbirth, HELLP syndrome, eclampsia, or even maternal death. The early diagnosis of PE is crucial to facilitate early intervention.

Traditional proteinuria testing, while simple and widely available, lacks sensitivity and specificity, posing diagnostic challenges. Addressing pre-analytical, analytical, and post-analytical limitations is essential to enhance the overall effectiveness and reliability of proteinuria testing. Although proteinuria is a symptom of PE, not all cases show protein in urine.[29] Proteinuria testing is therefore no longer required as a standard diagnostic protocol but is still valuable to assess kidney damage in PE.

Compared to proteinuria testing, miRNAs offer higher sensitivity and specificity, and appear early in circulation before proteinuria and hypertension manifests, making them promising biomarkers for PE diagnosis. The non-invasive detection in maternal circulation further enhances the potential use of miRNAs for routine screening during prenatal care. The use of miRNAs as biomarkers for the early diagnosis of PE improves the likelihood of predicting the condition compared to proteinuria testing.

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