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Increased circulating miR-155 identifies a subtype of preeclamptic patients

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Abstract

Introduction Preeclampsia is a common and severe pregnancy complication. The syndrome is highly heterogeneous, making accurate classification difficult, which is not conducive to find ways to predict and prevent this syndrome. Recently, we reported that high placental miR-155 defined a new subtype of preeclampsia. Here, we aimed to examine whether high maternal sero-miR-155 could be a marker to identify this subtype.

Methods To explore whether the patients with high sero-miR-155 no matter in first and third trimester, we conducted a case-control and a longitudinal cohort study. We measured the sero-miR-155 levels at first, second and third trimesters in all pregnant women. Then, using the 95th percentile (P95) of sero-miR-155 in controls as the cut-off value, we divided the preeclamptic patients into high sero-miR-155 group (\geq P95) and normal sero-miR-155 group ($<$ P95). We compared the difference of clinical manifestations between two groups and used t-distributed stochastic neighbor embedding (t-SNE) to evaluate whether the patients with high sero-miR-155 could be clustered as a subtype. Finally, we evaluated the predictive value of sero-miR-155 in the subtype.

Results The case-control study included 525 subjects (350 controls and 175 preeclampsia) and the longitudinal cohort study included 411 subjects (274 controls and 137 preeclampsia). Sero-miR-155 was significantly elevated in preeclampsia. Compared with preeclamptic patients with normal sero-miR-155 levels, the cases with high sero-miR-155 had significantly higher blood pressure and other severe preeclampsia-related complications. The incidences of HELLP syndrome [5.2% (5/96) vs. 0.9% (2/216), $p < 0.01$], visual disturbance [15.6% (15/96) vs. 4.6% (10/216), $p < 0.01$], hypertensive retinopathy [13.5% (13/96) vs. 3.2% (7/216), $p < 0.01$], and placenta abruption [7.3% (7/96) vs. 0.9% (2/216), $p < 0.01$] in patients with high miR-155 level were significantly increased. T-SNE analysis showed the patients with high sero-miR-155 were predominantly clustered on the left of the plot.

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Conclusions The patients with high sero-miR-155 exhibited more severe clinical manifestations and sero-miR-155 could be a biomarker to identify a subtype of preeclampsia with high sero-miR-155.

Keywords Preeclampsia, Heterogeneous, MiR-155, Serum, Subtype

Introduction

Preeclampsia is a common but poorly understood syndrome with adverse maternal and fetal outcomes [1–3]. The heterogeneity of this syndrome hinders our in-depth understanding of preeclampsia pathogenesis and prevents us from identifying and developing effective diagnostic and predictive tool for this disorder [4–8]. Therefore, discovering biomarkers to identify preeclampsia subtypes should be helpful to study their characteristics, and find targeted prevention methods, which might allow us to understand the syndrome step by step by addressing each subtype individually.

One of the well-studied and widely used biomarker for preeclampsia is placental growth factor (PlGF). It is commonly used as one of biomarkers for the screening and diagnosis of preeclampsia and placental dysfunction [9], and might be used to differentiate a subtype of preeclamptic patients [10]. However, as preeclampsia is a heterogeneous disorder, it remains unclear whether there exist other as yet unexamined biomarkers which may represent different subtypes of preeclampsia and therefore could also be utilized in preeclampsia assessment.

Previously, we and others reported that miR-155 derived from placenta is upregulated in severe preeclampsia [11, 12]. The upregulation of miR-155 could inhibit cytotrophoblast invasion and proliferation by targeting cyclin D1 [13]. Furthermore, placenta-derived miR-155 can be packed into exosomes which are delivered into the maternal circulation and inhibit vascular relaxation through endothelial nitric oxide synthase [12, 14–17]. Recently, we identified a new subtype of preeclampsia defined as high placental miR-155 [18]. In our previous study, we found that about 1/3 [30.77% (96/321)] patients had high placental miR-155. The patients with high placental miR-155 showed higher systolic blood pressure and proteinuria, higher rate of fetal growth restriction. The patients with high placental miR-155 also had higher incidence of HELLP syndrome, intensive care unit admission and gave birth earlier. The placentas from patients with high placental miR-155 presented severer vascular malperfusion. T-SNE analysis showed that patients with high placental miR-155 could be clustered as one group according to clinical manifestation or placental pathology. In addition, the placentas with high miR-155 exhibited specific placental transcriptomic signature (significant change in blood vessel development) and could be clustered by hierarchical clustering analysis [18].

Previously, we have found that sero-miR-155 was positively correlated with placental miR-155 [18]. In this study, to evaluate the sero-miR-155 levels throughout the whole pregnancy and whether sero-miR-155 could reflect this subtype of preeclampsia, we utilized case-control surveys and longitudinal cohort studies to explore the sero-miR-155 levels and the relationship between sero-miR-155 and this subtype of preeclampsia.

Materials and methods

Study design

This study included a case-control and a longitudinal cohort study. To explore whether that sero-miR-155 could reflect the subtype of preeclampsia with high placental miR-155, the case-control study was conducted between December 2017 and December 2020, including 525 subjects (350 controls and 175 preeclampsia), which had been previously published [18]. To explore whether sero-miR-155 was high before the onset of preeclampsia, we conducted a longitudinal cohort. The longitudinal cohort subjects were recruited between January 2017 and March 2020. The subjects attended our antenatal clinic from GW 11–13⁺6 for routine antenatal care and were followed up at GW 19–23⁺6, 30–33⁺6, and 35–38⁺6, and delivery respectively. Although the recruitment time of both studies overlapped for almost two years, the subjects from case-control study and longitudinal study were independent as the pregnant women in case-control study were referred to our hospital after 20 GW and whereas the subjects in longitudinal cohort were recruited as early as 11–13⁺6 GW, and when the patients in longitudinal cohort developed preeclampsia they were recorded into longitudinal study separated from the case-control group. The study was approved by the Ethics Committee of Nanjing Drum Tower Hospital (Approval Number 2016-113-01). Prior to participation, written informed consent was obtained from all participants.

Participants

The diagnostic criteria of preeclampsia were new onset hypertension (systolic blood pressure (SBP) sustained at ≥ 140 mmHg and/or diastolic blood pressure (DBP) sustained at ≥ 90 mmHg, or both) complicated with proteinuria and/or organ dysfunction after 20 weeks of gestation and the classifications of mild and severe preeclampsia, or early (< 34 weeks) and late onset pattern were based on the criteria proposed by International Society for the Study of Hypertension in Pregnancy (ISSHP) [19]. Controls were defined as uncomplicated normotensive

pregnant women who delivered healthy babies (excluded the infants with congenital anomalies). We excluded the patients with chronic hypertension, gestational hypertension, gestational diabetes mellitus, basic renal or heart disease, autoimmune diseases, evidence of infection and non-preeclamptic preterm birth and fetal growth restriction without preeclampsia.

Case-control study population

Some subjects referred from Grass-roots women's health care units for their routine antenatal cares after 20 gestational week (GW), who experienced normotensive uncomplicated pregnancies or developed preeclampsia later and the others were referred from primary hospitals with documented diagnosis of preeclampsia at presentation.

Longitudinal study population

The inclusion criteria were: age ≥ 18 years, singleton pregnancy with a live fetus, early gestational age determined by ultrasound scan. Exclusion criteria were: women with difficulty in communication or serious mental illness, GW above 13⁺₆ at first visit, fetal abnormality detected at GW 11–13⁺₆, and multiple pregnancy. In the prospective longitudinal cohort of pregnant women, they did not take oral aspirin. In the case-control study of pregnant women, 11 patients had taken oral aspirin 75 mg/d to 34 GW, and 21 cases in the control pregnant women also had taken aspirin 75 mg/d to 36 GW.

Maternal characteristics, obstetric and medical history, maternal weight and height, maternal blood pressure (BP) and proteinuria were measured and recorded in Viewpoint 6.0 (GE Healthcare GmbH, Germany) as previous report [20]. Written informed consents were obtained from the subjects.

Sample size

In the case-control study, a total of 175 preeclamptic patients and 350 normotensive uncomplicated pregnant women were included as previously mentioned [18]. In the longitudinal cohort, a total of 137 subjects suffered from preeclampsia and 274 normotensive uncomplicated pregnant women (1:2) were randomly chosen as controls by a senior biostatistician via SAS software. The exclusion was shown in Supplementary Fig. 1.

Serum samples

The serum was obtained according to standard protocols. Briefly, a total of 4mL blood samples were collected into standard Vacutainer tubes (367955, SST™ II Advance Plus Blood Collection Tubes, BD Vacutainer) and processed within 1 h by centrifugation at 3000 \times g for 15 min at 4 °C. The supernatants were quickly collected, aliquoted, and stored immediately at -80 °C in the Nanjing Drum Tower hospital Biobank of Jiangsu Provincial Science and Technology Resources (Clinical Resources) Coordination Service Platform within 2 h. Before use, sera samples were thawed on ice and centrifuged at 2000 \times g for 15 min. The serum was collected firstly and then used to measure the levels of sero-miR-155 or PlGF. In the case-control study, the gestational week at serum collection was 35.9 \pm 1.7 wks in control and 35.2 \pm 2.5 wks in preeclamptic patients (Table 1). In the longitudinal cohort, the gestational week at serum collection were GW 11–13⁺₆, GW 19–23⁺₆, GW 30–33⁺₆, and GW 35–38⁺₆.

In the clinical management of preeclamptic patients, the attending obstetricians and the patients were not aware of the test results of sero-miR-155 and PlGF. Therefore, the clinical treatment was not affected by the levels of sero-miR-155 and PlGF. The investigators who

Table 1 Clinical characteristics of subjects in the case-control study

Analysed items	Controls <i>n</i> = 350	PE <i>n</i> = 175	<i>p</i> . value
Maternal age, y	29.5 \pm 3.8	30.2 \pm 4.9	0.31
Pre-pregnancy BMI, kg/m ²	24.6 \pm 2.8	25.1 \pm 3.3	0.57
GW at serum collection, wks	35.9 \pm 1.7	35.2 \pm 2.5	0.34
GW at delivery, wks	39.1 \pm 2.3	35.8 \pm 3.6	< 0.01
IVF, <i>n</i> (%)	38 (10.9)	29 (16.6)	0.06
Multipara, <i>n</i> (%)	53 (15.1)	30 (17.1)	0.55
SBP, mmHg	114.6 \pm 8.9	156.2 \pm 13.8	< 0.01
DBP, mmHg	72.3 \pm 8.6	98.8 \pm 9.8	< 0.01
GW at BP measurement, wks	36.0 \pm 2.7	35.3 \pm 3.1	0.43
Proteinuria, mg/24 h	-	2307 [1080, 3776]	-
Birthweight, g	3684 \pm 443.5	2672 \pm 824.7	< 0.01

GW, gestational week; wks, weeks; BMI, body mass index; IVF, in-vitro fertilization; SBP, systolic blood pressure; DBP, diastolic blood pressure; PE, preeclampsia. The SBP and DBP were described as the highest blood pressure during admission

Continuous variables follow a normal distribution are expressed as mean \pm SD and Student's *t* tests are used for statistical analysis. Continuous variables follow an abnormal distribution are expressed as median [interquartile range]. Categorical variables are expressed as *n* (percentages) and Chi-square tests were used for statistical analysis

measured the levels of miR-155 were blinded to the clinical data and management.

Serum MiRNA isolation and measurement with real-time quantificational polymerase chain reaction (qPCR)

MiRNA was isolated from serum (200 μ L) using the miRNeasy Serum/Plasma Advanced Kit (217204, Qiagen, Germany) according to the manufacturer's protocol. cDNA for analyzing miRNAs was prepared from 10ng of total miRNA using a miRCURY LNA RT Kit (339340, Qiagen, Germany). qPCR was performed with a miRCURY LNA SYBR Green PCR Kit (339347, Qiagen, Germany) according to the manufacturer's instructions. The levels of miRNAs were analyzed by miRCURY LNA miRNA PCR Assay and el-miR-39 miRCURY LNA miRNA PCR Assay (339306, Qiagen, Germany). The relative expression of miR-155 was analyzed by the $2^{-\Delta\Delta CT}$ method, and miR-39 was used for normalization [12].

Measurement of maternal serum PlGF

The maternal serum PlGF was measured using the Cobas e602 system (Roche Diagnostics, Penzberg, Germany). When analyzing the expression pattern of PlGF in normotensive uncomplicated controls, we described the PlGF as original concentration. When analyzing the difference of PlGF between normotensive uncomplicated controls and preeclampsia cases, we converted the concentration to the multiples of the median (MOM) in the GW matched controls.

Statistical analysis

The continuous variables with normal distribution are presented as mean \pm SD. The continuous variables with non-normal distribution are presented as median (interquartile range). Student's *t* tests were used for continuous variables conforming to normal distribution. Wilcoxon rank sum tests were used for abnormal continuous variables. Chi-square tests were used for comparison of categorical variables and categorical variables were presented as proportions and counts. The screening value of sero-miR-155 for preeclampsia was tested by receiver operating characteristic (ROC) curve. The optimal cutoff point is the value corresponding to the maximum Youden index (Youden index = sensitivity + specificity - 1). Data analysis was performed using the SPSS software, version 22.0 (SPSS, Chicago, IL). Values of $p < 0.05$ were considered as statistical significance.

T-distributed stochastic neighbor embedding (t-SNE) was used to analyze the global relationships among all the patients based on the medical histories, clinical presentations, and pregnant outcomes, and to group the patients based on the levels of sero-miR-155 or PlGF.

Results

The expression patterns of sero-miR-155 of normal pregnant women

We investigated the expression changes of circulating miR-155 in pregnant women. The range of sero-miR-155 from normal pregnant women in case-control cohort (GW 35.9 ± 1.7) was from $3.4E-5$ to $4.9E-2$ ($2.5E-3$ [$1.1E-3$, $8.2E-3$], $P95 = 2.6E-2$) (Fig. 1A). In the sera of controls from longitudinal cohort, we found that sero-miR-155 levels from GW $35-38^{+6}$ were $3.0E-3$ [$1.3E-3$, $7.6E-3$] and $P95$ was $2.7E-2$, which was similar to that in case-control cohort. The sero-miR-155 levels in different gestational week from longitudinal cohort ranged from $9.8E-5$ to $9.9E-2$ ($3.2E-3$ [$1.4E-3$, $6.7E-3$], $P95 = 2.5E-2$) at GW $11-13^{+6}$, $9.7E-5$ to $9.2E-2$ ($3.3E-3$ [$1.2E-3$, $8.8E-3$], $P95 = 2.6E-2$) at GW $19-23^{+6}$, $4.3E-5$ to $6.0E-2$ ($3.5E-3$ [$1.2E-3$, $8.4E-3$], $P95 = 2.7E-2$) at GW $30-33^{+6}$. There was no significant difference between different gestational weeks (Fig. 1B).

The expression patterns of sero-miR-155 in preeclamptic patients

Among 175 preeclamptic patients from the case-control study, 116 cases were mild preeclampsia and 59 cases were severe preeclampsia, and 64 patients presented early-onset and 111 presented late-onset. The demographic and clinical characteristics in the case-control study subjects were in Table 1. The mean of sero-miR-155 level in preeclamptic patients was 1.39-fold higher than that in normal controls (Fig. 1A). However, this marker showed significantly variability, and in some cases, sero-miR-155 levels overlapped with those in controls, and the others with high sero-miR-155 ($\geq P95$), indicating that sero-miR-155 was heterogeneous in preeclampsia (Fig. 1C). In the longitudinal cohort, we also observed this situation (Fig. 1D). Among 137 preeclamptic patients in longitudinal cohort, 81 cases were mild and 56 were severe. Based on the GW of preeclampsia onset, 42 patients presented early-onset and 95 presented late-onset. The demographic and clinical characteristics of the longitudinal study subjects are shown in Table 2. Through calculations, we found that in the case-control study, 55/175 cases (about one-third) had high sero-miR-155 and in the longitudinal cohort, 41/137 cases (about one-third) also had elevated sero-miR-155.

The difference in clinical manifestations between preeclampsia with high and normal sero-miR-155

We combined the data of case-control study with the longitudinal study to analyze the clinical manifestations. There were totally 312 preeclamptic patients, of whom, 96 cases exhibited high sero-miR-155.

Compared to preeclamptic patients with normal miR-155 levels, the patients with high sero-miR-155 levels

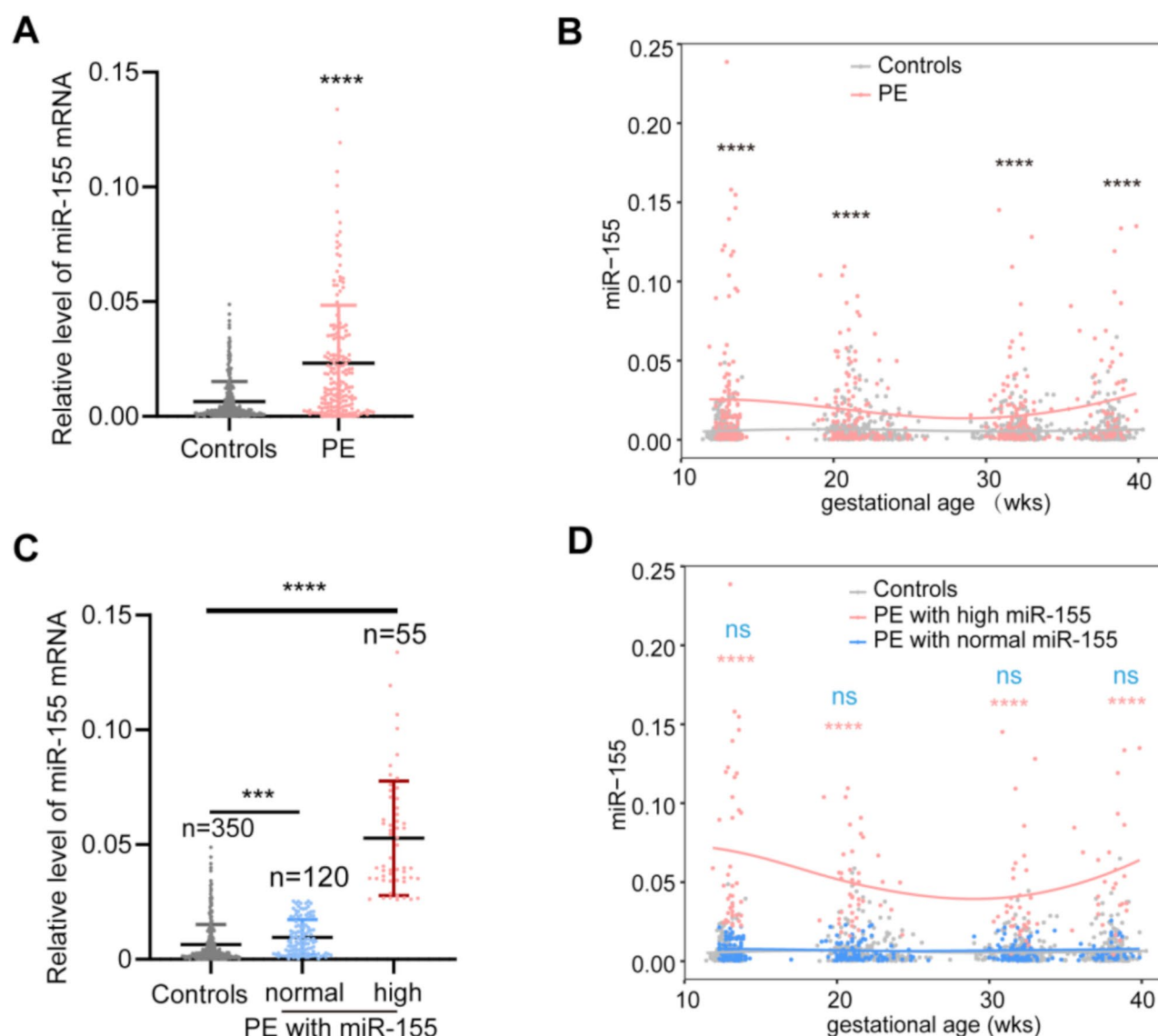


Fig. 1 Serum miR-155 increased in preeclamptic patients from the case-control and longitudinal study. **(A)** Serum miR-155 levels increased in preeclamptic patients compared to controls by qPCR analysis. **(B)** Serum miR-155 levels in preeclamptic patients were higher than those in controls from GW 11–13⁺⁶, 19–23⁺⁶, 30–33⁺⁶ and 35–38⁺⁶ respectively. **(C–D)** Based on the P95 value of controls, there were two groups: one group with high miR-155 levels (equal to or above P95), as preeclampsia with high miR-155; the other group with miR-155 below P95, as preeclampsia with normal miR-155 (GW at sero- sero-miR-155 measurement: Controls: 35.9 ± 1.7 wks, PE with high miR-155: 36.1 ± 2.8 wks, PE with normal miR-155: 35.0 ± 2.3 wks). Data were analyzed with Wilcoxon rank sum tests. Values are median (interquartile range). The curves were shown by curve fitting. PE, preeclampsia. ns, no significance; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.00001$. Blue represents the difference between controls and patients with normal miR-155 and pink represents the difference between patients with normal miR-155 and high miR-155 in Fig. 1D;

presented higher BP, especially in SBP (SBP: 161.4 ± 16.6 vs. 149.5 ± 10.7 mmHg, $p < 0.01$; DBP: 97.0 ± 12.5 vs. 94.4 ± 9.3 mmHg, $p < 0.05$) and the risk of severely elevated BP (SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg) was also significantly increased (63.5% vs. 25.0%, $p < 0.05$) (Table 3), therefore the number of patients who required urgent antihypertensive treatment (≥ 2 times) was increased (20.8% vs. 2.3%, $p < 0.01$) (Table 3). The level of urine protein in high sero-miR-155 group was significantly higher than that in normal sero-miR-155

group (median: 2032 vs. 2402 mg/24h, $p < 0.05$) (Table 3). In addition, patients with high sero-miR-155 level were more likely to develop early-onset preeclampsia than those with normal levels (52.1% vs. 25.9%, $p < 0.01$) (Table 3).

As to the maternal outcomes, there was no maternal death in this study. The patients admitted to the intensive care unit due to heart failure, pulmonary edema, acute renal failure and eclampsia accounted for 3.1% in high sero-miR-155 group, but none in patients with

Table 2 Clinical characteristics of subjects in the longitudinal study

Analyzed items	Controls <i>n</i> = 274	PE <i>n</i> = 137	<i>p</i> . value
Maternal age, y	29.9 ± 3.5	30.3 ± 3.8	0.43
Pre-pregnancy BMI, kg/m ²	23.3 ± 2.9	23.2 ± 3.4	0.54
GW at delivery, wks	39.0 ± 1.5	37.8 ± 1.9	< 0.05
IVF, <i>n</i> (%)	43 (15.7)	32 (23.4)	0.06
Multipara, <i>n</i> (%)	33 (12.0)	15 (10.9)	0.75
SBP, mmHg	118.0 ± 9.3	148.6 ± 12.4	< 0.01
DBP, mmHg	73.00 ± 8.4	93.9 ± 10.0	< 0.01
GW at BP measurement, wks	35.8 ± 1.3	35.6 ± 2.1	0.37
Proteinuria, mg/24 h	-	2800 [1530, 3890]	-
Birthweight, g	3675 ± 543.4	2809 ± 625.6	< 0.05

BMI, body mass index; GW, gestational week; IVF, in-vitro fertilization; SBP, systolic blood pressure; DBP, diastolic blood pressure; PE, preeclampsia. The SBP and DBP were described as the highest blood pressure during admission

Continuous variables follow a normal distribution are expressed as mean ± SD and Student's *t* tests are used for statistical analysis. Continuous variables follow an abnormal distribution are expressed as median [interquartile range]. Categorical variables are expressed as *n* (percentages) and Chi-square tests were used for statistical analysis

normal miR-155 level ($p < 0.01$) (Table 3). Compared with patients with normal miR-155 levels, the incidences of HELLP syndrome (5.2% vs. 0.9%, $p < 0.01$), visual disturbance (15.6% vs. 4.6%, $p < 0.01$), hypertensive retinopathy (13.5% vs. 3.2%, $p < 0.01$), and placenta abruption (7.3% vs. 0.9%, $p < 0.01$) in patients with high sero-miR-155 level were significantly increased (Table 3).

Regarding fetal outcomes, there was no perinatal death in this study. The rate of neonatal intensive care unit admission (NICU) in high sero-miR-155 group was significantly higher than that with normal sero-miR-155 group (24.0% vs. 5.1%, $p < 0.05$) (Table 3). The mean GW of pregnancy termination was lower than that of the patients with normal sero-miR-155 (35.5 ± 3.6 vs. 37.2 ± 2.8 weeks, $p < 0.01$) and the rate of birthweight < 3th percentile was significantly increased (21.9% vs. 4.6%, $p < 0.01$) in high sero-miR-155 group compared to the patients with normal sero-miR-155 (Table 3).

The expression of PlGF levels and clinical manifestations of low PlGF in preeclampsia

As serum PlGF is an important biomarker for preeclampsia, we also measured the PlGF levels in the subjects. In 274 normotensive pregnant women from longitudinal cohort, the median serum PlGF level was 51.5 pg/mL at GW 11–13⁺, increased obviously at GW 19–23⁺ (375.2 pg/mL, $p < 0.0001$) and to peak at GW 30–33⁺ (548.3 pg/mL, $p < 0.0001$) and then decreased to 226.2 pg/mL at GW 35–38⁺ (Supplementary Fig. 2A). The PlGF levels significantly decreased in preeclamptic patients from both case-control study and longitudinal cohort study, but also showed overlap with the controls, which represented the heterogeneity of PlGF in the preeclampsia (Supplementary Fig. 2B–C). We also set P5 of PlGF levels in controls as a cutoff value and divided the subjects into two groups: one with low PlGF levels and the other with

normal PlGF levels (Supplemental Fig. 2D–E). We found that there was a total of 97 patients with low sero-PlGF in the case-control study and longitudinal cohort.

Compared to preeclamptic patients with normal PlGF levels, the patients with low sero-PlGF levels presented also higher BP, especially in SBP (SBP: 154.9 ± 15.9 vs. 150.6 ± 10.1 mmHg, $p < 0.01$; DBP: 96.5 ± 11.5 vs. 94.1 ± 9.5 mmHg, $p < 0.05$) (Supplemental Table 1). However, the risk of severely elevated BP (SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg) was not increased significantly (38.1% vs. 36.3%, $p > 0.05$) (Supplemental Table 1). The other clinical manifestations with dramatic differences between patients with and without high miR-155 level, were not observed between patients with normal and low PlGF level (Supplemental Table 1). In addition, in patients with low PlGF, there were more neonates who required admission in NICU (16.5% vs. 8.4%, $p < 0.05$) and who had birthweight < 3th percentile (15.5% vs. 7.4%, $p < 0.01$) than those in the group with normal PlGF (Supplemental Table 1).

The preeclamptic patients with high sero-miR-155 level can be clustered as one group

To further investigate whether patients with high sero-miR-155 exhibited similarities in the medical history, clinical presentations and pregnant outcomes, we conducted t-SNE analysis on a total 312 patients (including cases from the case-control study and the longitudinal cohort). The results showed that the patients with high sero-miR-155 pattern were predominantly clustered on the left of the plot, whereas the patients with normal miR-155 level showed dispersed distribution (Fig. 2). On the contrary, the patients with low or high PlGF levels did not show clear clusters (Supplementary Fig. 2F).

Table 3 The medical history, clinical presentations and pregnant outcomes between patients with high miR-155 and normal miR-155

Analyzed items	PE		p.value
	Normal miR-155 n = 216	High miR-155 n = 96	
Maternal age, y	30.5 ± 4.4	30.3 ± 4.8	0.35
Pre-pregnancy BMI, kg/m ²	24.4 ± 3.5	24.1 ± 3.2	0.23
IVF, n (%)	41 (19.0)	20 (20.8)	0.70
Abortion history, n (%)	27 (12.5)	31 (32.3)	< 0.05
Multipara, n (%)	30 (13.9)	15 (15.6)	< 0.01
PE history, n (%)	22 (10.2)	12 (12.5)	0.55
Maternal assessment and diagnosis			
SBP, mm Hg	149.5 ± 10.7	161.4 ± 16.6	< 0.01
DBP, mm Hg	94.4 ± 9.3	97.0 ± 12.5	< 0.05
SBP ≥ 160 and/or DBP ≥ 110 mm Hg, n (%)	54 (25.0)	61 (63.5)	< 0.01
Proteinuria, mg/24 h	2400 [1030, 3560]	3032 [1630, 3870]	< 0.05
Serum creatinine, μM	50.4 ± 14.6	50.5 ± 9.5	0.52
Serum uric acid, μM	401.3 ± 91.9	380.8 ± 88.4	0.46
AST, U/L	21.3 ± 13.2	31.1 ± 30.6	< 0.01
ALT, U/L	19.1 ± 7.9	23.8 ± 20.2	0.06
Platelet, ×10 ⁹ /L	178.0 ± 52.0	159.2 ± 61.3	< 0.01
Platelet < 100 × 10 ⁹ /L	7 (3.2)	17 (17.7)	< 0.01
Requiring urgent antihypertensive therapy (≥ 2 times), n (%)	5 (2.3)	20 (20.8)	< 0.01
HELLP, n (%)	2 (0.9)	5 (5.2)	< 0.01
Visual disturbance, n (%)	10 (4.6)	15 (15.6)	< 0.01
Hypertensive retinopathy, n (%)	7 (3.2)	13 (13.5)	< 0.01
Placental abruption, n (%)	2 (0.9)	7 (7.3)	< 0.01
MICU, n (%)	0 (0.0)	3 (3.1)	< 0.01
The early-onset PE, n (%)	56 (25.9)	50 (52.1)	< 0.01
Pregnancy outcome			
GW at delivery, wks	37.2 ± 2.8	35.5 ± 3.6	< 0.01
Male infant, n (%)	111 (51.4)	51 (53.1)	0.78
Birthweight < 3th, n (%)	10 (4.6)	21 (21.9)	< 0.01
Apgar ≤ 7 at 1 min, n (%)	5 (2.3)	6 (6.3)	0.16
Apgar ≤ 7 at 5 min, n (%)	5 (2.3)	3 (3.1)	0.98
NICU, n (%)	11 (5.1)	23 (24.0)	< 0.01

PE, preeclampsia; BMI, body mass index; IVF, in-vitro fertilization; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MICU, maternal intensive care unit admission; GW, gestational week; NICU, neonatal intensive care unit admission. The SBP and DBP were described as the highest blood pressure during admission

Continuous variables follow a normal distribution are expressed as mean ± SD and Student's t tests are used for statistical analysis. Continuous variables follow an abnormal distribution are expressed as median [interquartile range] and Wilcoxon rank sum tests are used for statistical analysis. Categorical variables are expressed as n (percentages) and Chi-square tests were used for statistical analysis

Sero-miR-155 could identify this subtype of preeclampsia in the first trimester

To investigate when increase of sero-miR-155 took place in the preeclampsia with high sero-miR-155, we explored the expression of sero-miR-155 in different gestational weeks before onset of preeclampsia with high sero-miR-155. As shown in Fig. 3A, in patients with high sero-miR-155, the increase of sero-miR-155 occurred as early as 26~30 weeks before preeclampsia-onset and namely, the value of sero-miR-155 detected in first trimester can be used to identify the subtype of preeclampsia. ROC curve was constructed using the values of 11–13⁺ gestational weeks and showed that the area under the ROC curve for screening all preeclampsia patients (including

normal sero-miR-155 and high sero-miR-155) was 0.690 (95%CI 0.634–0.746). The optimal cut-off point was 0.0068 with 54% sensitivity and 77% specificity. The positive value of sero-miR-155 for preeclamptic patients was 75.93% and negative predictive value was 73.11%. however, for screening preeclampsia patients with high sero-miR-155, the area under the ROC curve was 0.98 (95%CI 0.969–0.994) (Fig. 3B). The optimal cut-off point was 0.0224 with 100% sensitivity and 95.3% specificity. The positive value of sero-miR-155 for preeclamptic patients with high sero-miR-155 was 75.93% and negative predictive value was 100%.

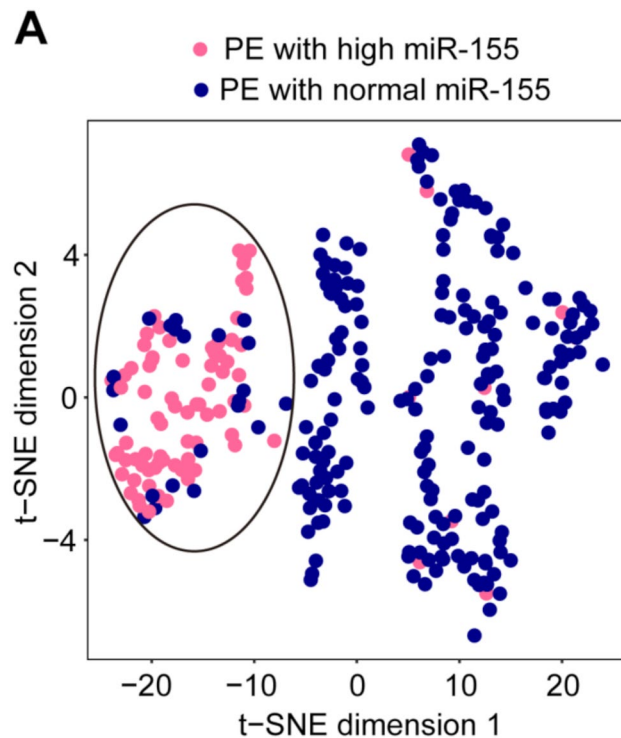


Fig. 2 Patients with high miR-155 levels presented a subtype of PE. (A) Preeclamptic patients with high miR-155 were predominantly clustered on left of the plot as analyzed by t-SNE analysis. PE, preeclampsia

Discussion

In this study, we first evaluated the sero-miR-155 levels at different gestational week, and found that there was no significant difference from first trimester to third trimester. There was about 30% preeclamptic patients

presented remarkable elevation of sero-miR-155 both in a case-control cohort and a longitudinal cohort. The patients with high sero-miR-155 had more severe clinical manifestations and more adverse maternal and fetal outcomes, which was similar to the patients with high placental miR-155 [18]. Noteworthy, the patients with high sero-miR-155 could be well clustered as a group. Moreover, for patients with high sero-miR-155, the sero-miR-155 increased as early as 26~30 weeks before the onset of preeclampsia and the area under the ROC curve was 0.98, indicating that the value of sero-miR-155 in the first trimester could identify the preeclamptic patients with high sero-miR-155.

MiR-155 is a pleiotropic microRNA and played a regulatory role in cell growth, invasion, migration, inflammation and angiogenesis [21]. Pineles et al. firstly reported that the expression of miR-155 was increased in placentas of preeclamptic patients with small-for-gestational age (SGA) neonates [11]. We reported that placental miR-155 was significantly increased in severe preeclamptic patients [12], and led to insufficient remodeling of spiral arteries through down-regulating cysteine-rich 61 protein (CYR61) in trophoblasts [12] and vascular endothelial growth factor C (VEGFC) in natural killer cells [22]. In addition, we and others uncovered that upregulation of miR-155 can suppressed vascular relaxation by inhibiting the expression of endothelial nitric oxide synthase (eNOS) in endothelial cells [14, 15, 17]. Recently, miR-155 regulated M2 macrophage polarization in preeclampsia [23] and induced the formation of proteinuria by increasing interleukin-17 production in CD4⁺ T cells during late onset of preeclampsia [24]. Moreover,

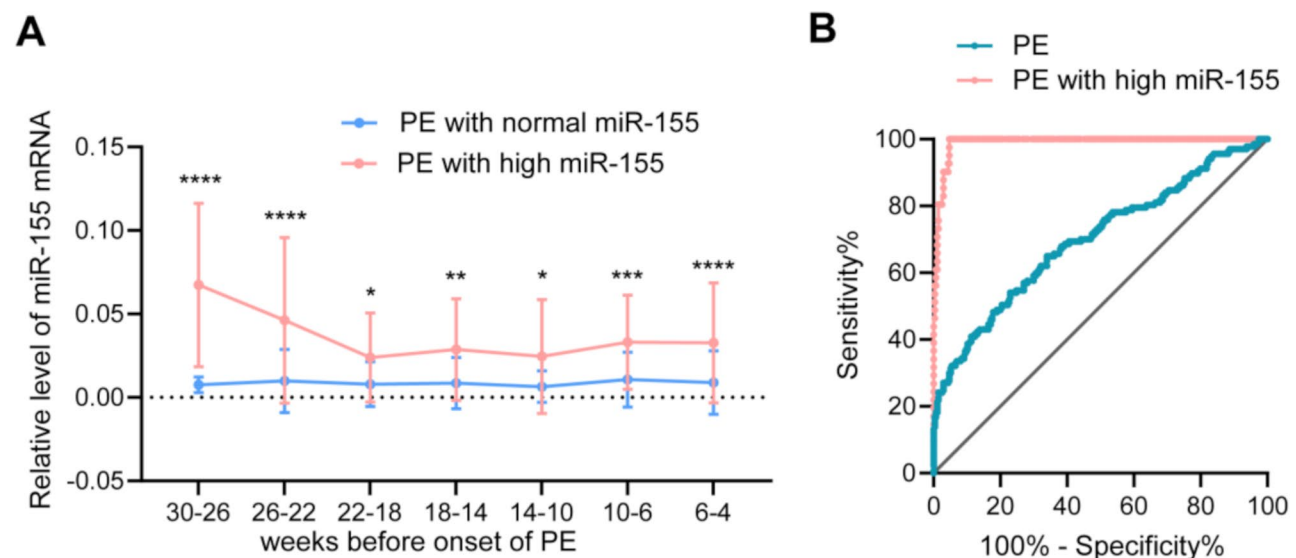


Fig. 3 The predictive value of sero-miR-155 for preeclampsia with high miR-155. (A) The sero-miR-155 levels according to the number of weeks before the onset of preeclampsia with normal miR-155 and high miR-155. (B) ROC curve analysis for sero-miR-155 between the patients of preeclampsia or preeclampsia with high miR-155 and healthy pregnancies

methylation mediated silencing of miR-155 could suppresses the development of preeclampsia [25]. Some studies also have investigated that sero-miR-155 was related with severity of preeclampsia [26, 27], which indicated that there may be existed a subtype with high sero-miR-155. In addition, decreased CYR61 was related to recurrent pregnancy loss [28], we could also explore the relationship between miR-155 and recurrent pregnancy loss in the future.

The limitation of our previous finding is that placenta must be obtained after childbirth, which makes it difficult for us to identify this subtype early. Could over-expression of sero-miR-155 reflect this subtype? In our study, we also divided the preeclamptic patients into two groups based on the P95 of sero-miR-155 levels. The patients with high sero-miR-155 exhibited severer clinical manifestations, such as higher blood pressure, needed more emergency antihypertensive therapy and significant tendency to develop early-onset preeclampsia, which were similar to the patients with high placental miR-155. Moreover, these patients also had similar maternal and fetal outcomes, such as higher incidence of HELLP syndrome, higher rate of maternal intensive care unit and NICU. Furtherly, we used t-SNE analysis to cluster all preeclamptic patients and found that patients with high sero-miR-155 were mostly clustered on the left of the plot and the patients with normal sero-miR-155 appeared scattered. The homogeneity of these clinical manifestations and our t-SNE analysis indicated that patients with high sero-miR-155 could be viewed as a subtype of preeclampsia.

Recently, we focused more on early identification of preeclampsia for better management of these patients. In patients with high sero-miR-155, the increase of sero-miR-155 took place at GW11-13⁺⁶ and maintained high level across pregnancy. Besides, for preeclamptic patients with high sero-miR-155, we found that sero-miR-155 increased as early as 26~30 weeks before the onset of preeclampsia in these patients, which provided a basis for future evaluation of preventive methods. Furtherly, we evaluated the screening value of sero-miR-155 for the subtype of preeclampsia with high sero-miR-155. In the first trimester, a total of 54 pregnant women showed high sero-miR-155 levels and among which, 41 pregnant women eventually developed preeclampsia with high sero-miR-155. The area under the ROC curve in preeclamptic patients with high sero-miR-155 reached to 0.98. These results showed that sero-miR-155 is a good biomarker for identifying the subtype of patients with high sero-miR-155.

PIGF is an angiogenic factor, with known low serum level in placenta-mediated disease especially in preeclampsia [9]. Chappell LC et al. reported that in women presenting before 35 weeks' gestation with suspected

preeclampsia, low PIGF has high sensitivity for the diagnosis of preeclampsia within 14 days [29]. Recently, on behalf of the PARROT trial group, Duhig et al. reported that in a real world setting the availability of PIGF test results substantially reduces the time to clinical confirmation of preeclampsia [30].

In this study, we also demonstrated that PIGF in preeclampsia was low overall, with some of the patients below the P5 in normal controls and the greatest difference occurred after 20 GW, which was similar to the findings of previous reports [10, 31]. However, although the patients with low PIGF level showed more severe hypertension and more FGR, the t-SNE analysis showed that those patients could not be well clustered as a group (Supplementary Fig. 2F), suggesting that the clinical heterogeneity may exist in the patients with low PIGF level. This study's results suggested that clinical heterogeneity may exist in the patients with low PIGF levels, although the sero-PIGF showed better predictive value than sero-miR-155. Sero-miR-155 can identify a specific subtype of pre-eclampsia, while PIGF was a better predictor for pre-eclampsia.

A major strength of this study is our well-defined cohort, including a longitudinal study related to preeclampsia and a preeclampsia case-control study and the findings in the case-control study was reproduced in longitudinal study. We demonstrate that sero-miR-155 had the potential as a predictive biomarker for the subtype of patients with high sero-miR-155. This is a step forward in the development of stratified medicine in obstetrics where precision medicine could be applied.

However, there were several limitations in the present study. First, this study was performed just in one hospital, and the results were not validated by other centers. Second, the correlation of high sero-miR-155 level with other parameters for classification of preeclampsia remains clarified, such as "early-" and "late-" onset, "mild" and "severe", and "maternal" and "placental" preeclampsia. Thirdly, considering that soluble fms-like tyrosine kinase 1 (sFlt-1) /PIGF ratio was more commonly applied in the prediction of preeclampsia, we need to evaluate sFLT-1/PIGF ratio in the prediction of preeclampsia with high sero-miR-155.

Collectively, there is a subtype of preeclampsia with high sero-miR-155, which could reflect the subtype of high placental miR-155. Sero-miR-155 in the first trimester could help to identify this subtype of preeclampsia.

Abbreviations

BP	Blood pressure
DBPs	Diastolic blood pressures
GW	Gestational week
ISSHP	International Society for the Study of Hypertension in Pregnancy
miR-155	microRNA-155
MOM	Multiples of the median
PIGF	Placental growth factor

qPCR Quantificational polymerase chain reaction
SBPs Systolic blood pressures
t-SNE t-distributed stochastic neighbor embedding

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12884-025-07424-3>.

Supplementary Material 1

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Not Applicable.

Author contributions

Z.W., M.Z., and H.D. analyzed the data, and wrote the manuscript. Y.W., H.T., Y.W., and C.C. performed the investigation and data curation. N.G. and Q.W. were responsible for the validation of any uncertainties within the data. The original draft of the article was written by Z.W., and reviewed and edited by Y.Z., G.Z., and Y.H. All aspects of this research were supervised by Y.Z., G.Z., and Y.H. All authors reviewed the manuscript.

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Data availability

The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments. It was approved by the ethics committee of Nanjing Drum Tower Hospital (2016-113-01). Written informed consent was gained from all patients; all participants were informed that they could withdraw at any point during the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Disclosure statement

No conflict of interest was reported by the author(s).

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