Int J Clin Exp Med 2019;12(6):7383-7391
www.ijcem.com /ISSN:1940-5901/IJCEM0091781

Original Article

Clinical significance of miR-133a and miR-206 in pregnant women with preeclampsia and correlation with pregnancy outcomes

Rong Ma[1*], Yan Lu[2*], Chunjiang Dou[1], Qiaoling Gu[1]

[1]*Medical College, Northwest Minzu University, Lanzhou, Gansu, China;* [2]*B Ultrasound Room, Gansu Provincial Maternity and Childcare Hospital, Lanzhou, Gansu, China.* [*]*Equal contributors and co-first authors.*

Received January 24, 2019; Accepted March 11, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Objective: The aim of the current study was to investigate the clinical significance of peripheral blood miR-133a and miR-206 in pregnant women with preeclampsia (PE), examining correlation with pregnancy outcomes. Methods: A prospective cohort study design was used for 89 patients with PE. A total of 70 pregnant women with no evident anomalies detected during a physical examination were enrolled in the control group. Based on differences in pregnancy outcomes of patients with PE, they were further divided into the normal pregnancy subgroup (n=55) and abnormal pregnancy subgroup (n=34). Peripheral blood samples were collected to determine relative expression levels of miR-133a and miR-206 using quantitative real-time polymerase chain reaction (qRT-PCR). Pearson's analysis was performed to identify correlation between miR-133a and miR-206, while multifactor logistic regression analysis was used to determine risk factors of poor pregnancy outcomes. Results: miR-133a expression was significantly upregulated in the control group, compared to that in the observation group (P<0.05). Similar results were observed for expression of miR-206 (P<0.05). Expression of miR-133a and miR-206 was significantly higher in the normal pregnancy subgroup than in the abnormal pregnancy subgroup (P<0.05). Multifactor logistic regression analysis revealed miR-133a (OR: 1.204, 95% CI: 1.058-1.362) and miR-206 (OR: 1.096, 95% CI: 1.029-1.155) as independent risk factors of poor pregnancy outcomes. Pearson's correlation analysis also indicated a positive correlation between miR-133a and miR-206 expression in the peripheral blood of subjects in the two groups (r=0.543, P<0.001). Conclusion: miR-133a and miR-206 expression is downregulated in the peripheral blood of patients with PE, possibly correlating with poor pregnancy outcomes associated with PE.

Keywords: miR-133a, miR-206, preeclampsia, pregnancy outcomes

Introduction

Preeclampsia (PE) has an incidence rate between 2% and 8%, worldwide [1, 2], resulting in the death of 70,000 pregnant or maternal women and 500,000 perinatal infants. Pregnant women with PE usually show hypertension, proteinuria, and systemic multiorgan dysfunction [3]. Existing evidence [4] indicates that nearly 10% to 20% of pregnant women with gestational hypertension develop PE. PE patients have been associated with spasming of the small vessels, inducing endothelial injury and focal ischemia. This eventually results in the death of pregnant women and perinatal infants [5]. Currently, the pathogenesis of PE remains unknown. However, placental dysfunction has been widely recognized as a major factor contributing to endothelial injury and inflammatory response. It has been recognized as the major cause of PE development [6]. In addition, PE development has been correlated with cardiovascular disease, obesity, and other complications in pregnant women.

With advancement in medical techniques and progress made in PE-related studies, studies on PE conducted from the molecular viewpoint have become a hot topic [7]. MicroRNAs (miRs), specific non-coding single-chain RNA about 21 nucleotides in length, can degrade the mRNA of target genes by complementing the 3'-UTR region of target genes, inhibiting expression of target genes [8, 9]. At present, more than 3,700 miRs have been identified. Bioinformatic analysis shows that more than 30% of the genes in

Table 1. Primer sequences

Gene	Upstream primer	Downstream primer
miR-133a	5iR-133	5iR-133
miR-206	5iR-206	5iR-206
U6	56R-206	56R-206

humans are regulated by miRs. Thus, miRs play key roles in several biological processes, including growth, development, cell proliferation and apoptosis, cell differentiation, and development and progression of tumors [10]. Pineles et al. [11] reported abnormal expression of miRs in the placenta and correlation between miR and PE development in pregnant or maternal women. Moreover, miR-133a, a subtype of miR-133, and miR-206, are key members of the myomiR family. Members of the myomiR family [12] may perform regulatory roles in heart disease, but correlation of miR-133a and miR-206 with development of PE remains unknown.

Therefore, the current study investigated expression of miR-133a and miR-206 in the peripheral blood of pregnant women with PE, examining pregnancy outcomes of the fetus and providing a reference for clinical diagnosis and treatment of PE.

Methods and materials

Clinical data of subjects

The current prospective study enrolled 89 PE patients admitted to the hospital for treatment (observation group). Simultaneously, 70 pregnant women with no evident anomalies detected during physical examinations were enrolled in the control group. In the control group, subjects were aged 24-32 years, with an average age of 26.81±4.25 years. In the observation group, subjects were aged 25-36 years, with an average age of 27.25±5.12 years. Patients in the observation group conformed to the diagnostic criteria of PE set by the International Society for the Study of Hypertension in Pregnancy (ISSHP) [13]. Study protocols were approved by the Ethics Committee and all patients and families provided informed consent.

Inclusion and exclusion criteria for all subjects

Inclusion criteria: Subjects aged above 18 years; Subjects with a gestational period longer than 20 weeks; Subjects with no congenital immunodeficiencies, physical disabilities, or cognitive dysfunction; Subjects willing to cooperate with treatment protocols and follow-up.

Exclusion criteria: Subjects with primary hypertension, diabetes mellitus, malnutrition, prenatal infections, or fetal chromosomal abnormalities; Subjects with one or more malignant tumors; Subjects with other complications developed during the pregnancy period.

Major reagents and instruments

TRIzol Reagent (15596018, Invitrogen, USA); TransScript Green miRNA Two-Step qRT-PCR SuperMix (AQ202-01, TransGen Biotech, Beijing, China); miR-133a and miR-206 primers (**Table 1**) (Shanghai GenePharma Co., Ltd, Shanghai, China); ABI7500 PCR apparatus (ABI, USA); Ultraviolet spectrometer (EU-2800RS, Shanghai Onlab Instrument Co., Ltd).

Detection of miR-133a and miR-206

At 20 weeks of gestation, 3-mL blood samples were collected from subjects in the two groups. They were centrifuged at 3,000 rpm for 10 minutes at 4°C to isolate the serum, which was then stored at -80°C for subsequent experiments. TRIzol Reagent was used to extract total RNA in the serum of patients, according to manufacturer instructions. Purities and concentrations of the isolated RNA samples were measured using the ultraviolet spectrometer. Moreover, agarose gel electrophoresis was performed to determine the integrity of RNA. In accordance with instructions, reverse transcription was performed using isolated total RNA. Amplification was carried out with the ABI 7500 PCR apparatus, using the following PCR reaction system: 0.4 μL upstream primer + 0.4 μL downstream primer + 1 μL cDNA + 0.4 μL passive reference dye (50×) + 10 μL 2× TranStart® Tip Green qPCR SuperMix. The PCR reaction mixture was then diluted to 20 μL using ddH_2O. PCR protocol included pre-denaturation at 94°C for 30 seconds, 40 cycles of denaturation at 94°C for 5 seconds, and annealing and extension at 60°C. Expression of target miRs was normalized to that of U6 and data analysis was performed with the $2^{-\Delta\Delta Ct}$ method. For each sample, three replicate wells were set. The experiment was performed in triplicate.

Table 2. Comparison of clinical data between two groups [n (%)]

Factor	Control group (n=70)	Observation group (n=89)	X^2/t value	*P* value
Age (age)	26.81	27.25	0.659	0.511
Gestational age of delivery	38.95	36.58	9.738	<0.001
BMI during delivery (kg/m^2)	27.05	28.66	3.469	0.001
MABP (mmHg)	86.79	135.96	27.258	<0.001
Neonatal weight (g)	2425.33	3586.47	10.907	<0.001
Intraoperative bleeding volume (mL)	76.88	132.81	37.225	<0.001
Urine protein (g/L)	-	3.58	24.943	<0.001
Smoking history			0.480	0.489
Yes	20 (28.57)	30 (33.71)		
No	50 (71.43)	59 (66.29)		
History of alcoholism			0.146	0.703
Yes	3 (4.29)	5 (5.62)		
No	67 (95.71)	84 (94.38)		
Degree of education			0.155	0.694
> Junior middle school	50 (71.43)	61 (68.54)		
≤1 (68.54) school	20 (28.57)	28 (31.46)		
Domicile			0.351	0.554
Village	19 (27.14)	28 (31.46)		
City	51 (72.86)	61 (68.54)		

Evaluation of pregnancy outcomes

According to pregnancy outcomes, patients in the observation group were further divided into the normal pregnancy subgroup and abnormal pregnancy subgroup, in accordance with the following criteria [14]: (1) Perinatal death (intrauterine death, induced labor, or neonatal death); (2) Neonates admitted to the NICU after birth; (3) Neonates with an extremely low birth weight; (4) Neonates with Apgar scores <7 within 5 minutes after birth; (5) Fetuses with limited intrauterine growth; and (6) Presence of neonatal respiratory distress syndrome.

Outcome measures

Major outcome measures: Relative expression levels of miR-133a and miR-206 were observed in the peripheral blood of subjects in the two groups. According to pregnancy outcomes, changes in relative expression levels of miR-133a and miR-206 were measured in the peripheral blood of subjects.

Secondary observation indices: Correlation analysis between miR-133a and miR-206 levels of patients in the observation group was performed. Risk factors of poor pregnancy outcomes were determined using multifactor logistic regression analysis.

Statistical methods

SPSS 20.0 software (IBM, New York, USA) was used for statistical analysis of collected data and images were prepared using GraphPad Prism 7. Enumeration data, presented as rates (%), were compared using Chi-squared tests. Measurement data are expressed as mean ± standard deviation and were compared with independent *t*-tests. Correlation between miR-133a and miR-206 was analyzed with Pearson's correlation analysis. Risk factors for poor pregnancy outcomes, including fetal distress, fetal growth restriction, stillbirth, 1- and 5-minute Apgar scores, and neonatal body weights were identified with multivariate logistic regression analysis. $P<0.05$ suggests that differences are statistically significant.

Results

Comparison of clinical data of subjects in the two groups

Clinical data analysis of subjects in the two groups indicated that differences in age, smoking history, drinking history, education, and residence were not statistically significant ($P>0.05$). However, differences in gestational period, body mass index (BMI) at delivery, mean arterial pressure (MAP), neonatal weight, amount of intraoperative bleeding, and urinary protein levels showed statistical significance ($P<0.05$; **Table 2**).

Relative expression of miR-133a and miR-206 in the peripheral blood of subjects in the two groups

Measurement of relative expression levels of miR-133a and miR-206 in the peripheral blood of subjects in the two groups showed that miR-133a expression was significantly upregulated in the control group, compared to that in the

Table 3. Relative expression of miR-133a and miR-206 in the peripheral blood of two groups of subjects

Group	miR-133a	miR-206
Control group (n=70)	1.027	1.015
Observation group (n=89)	0.549	0.625
t value	21.794	16.670
P value	<0.001	<0.001

observation group ($P<0.05$). Similar results were observed for expression of miR-206 ($P<0.05$; **Table 3** and **Figure 1**).

Relative expression of miR-133a and miR-206 in the peripheral blood of subjects in normal and abnormal pregnancy groups

According to differences in the pregnancy outcomes, patients were further divided into the normal pregnancy subgroup (n=55) and abnormal pregnancy subgroup (n=34). Comparison of relative expression levels of miR-133a and miR-206 in the peripheral blood of subjects in the two groups showed that expression of both miR-133a and miR-206 was higher in the normal pregnancy subgroup than in the abnormal pregnancy subgroup. Differences were statistically significant ($P<0.05$; **Table 4** and **Figure 2**).

Single-factor analysis for subjects in normal and abnormal pregnancy groups

Single-factor analysis revealed that differences in age, mean arterial pressure (MAP), and urinary protein levels ($P<0.05$) were statistically significant between normal and abnormal pregnancy subgroups. However, differences in body mass indexes of patients at the time of delivery, as well as weights of the neonates and intraoperative bleeding amounts, were not statistically significant ($P>0.05$; **Table 5**).

Multifactor logistic regression analysis

Multifactor logistic regression analysis revealed miR-133a (adjusted OR: 1.204, 95% CI: 1.058-1.362) and miR-206 (adjusted OR: 1.096, 95% CI: 1.029-1.155) as independent risk factors of poor pregnancy outcomes (**Tables 6** and **7**).

Correlation analysis between relative expression of miR-133a and miR-206 in the peripheral blood of subjects in the two groups

Pearson's correlation analysis was performed, identifying correlation between relative expression levels of miR-133a and miR-206 in the serum of patients in the observation group. Results indicated a positive correlation between miR-133a and miR-206 expression in the peripheral blood of subjects in the two groups ($r=0.543$, $P<0.001$; **Figure 3**).

Discussion

PE, a common gynecological disease, is a severe complication during the gestational period in pregnant or maternal women. PE is mainly characterized by increased diastolic and systolic pressure, 24 hour proteinuria ≥300 mg, and maternal organ dysfunction after 20 gestational weeks [15]. According to epidemiological investigations [16, 17], about 1 million pregnant or maternal women die from PE or pregnancy-related complications every year, worldwide, especially in moderate- or low-income countries. In China, incidence of PE is 9.4% among pregnant or maternal women. Moreover, a high mortality rate, second only to that with postpartum hemorrhaging, has been associated with PE. In the absence of any appropriate or in-time treatment, PE may become latent, developing into eclampsia and severely threatening the health and life of both the pregnant woman and the fetus [18]. Various factors, including PE history, hypertension or family history of hypertension, positive response to antiphospholipid antibodies, multiple pregnancies, aged pregnant women, diabetes mellitus, renal diseases, obesity, and secondary pregnancy, have been identified to be involved in the pathogenesis of PE [19]. Due to implementation of the two-child policy, the number of aged pregnant women has increased, with an increasing trend in incidence of PE. However, there are effective methods for prophylaxis, diagnosis, and treatment of PE.

Scholars have speculated that there is a close correlation between incidence of PE and placenta. With the delivery of the placenta in the 3rd stage of labor, PE can be visibly alleviated. Patients may even recover without any intervention [20]. The placenta is a complicated structure that mainly nourishes embryonic cells. Any anomaly will contribute to decreased blood perfusion, ischemia, and anoxia of the placenta, resulting in the delivery of some factors into the blood circulation, even to vascular endothelial cells [21]. In addition to correlation with the placenta, development of PE has been associated with nutrition, inflammation, insulin

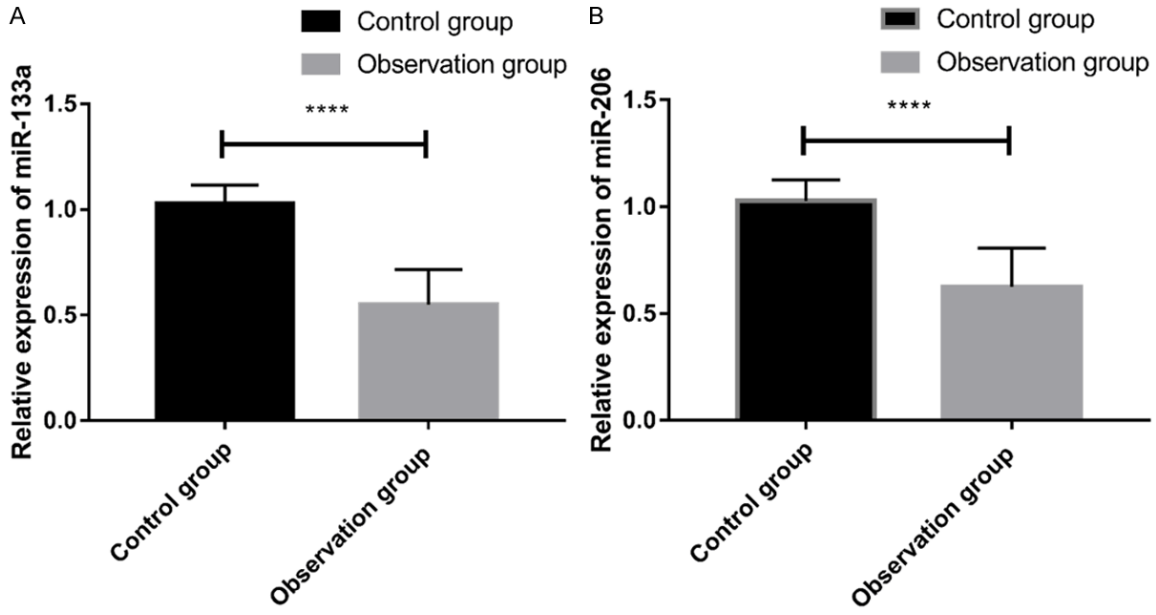

Figure 1. Relative expression of miR-133a and miR-206 in the peripheral blood of patients in the observation group and control group. A. Expression of miR-133a in the peripheral blood of the two groups of patients detected by qRT-PCR. miR-133a expression was significantly higher in the control group than in the observation group. B. Expression of miR-206 in the peripheral blood of the two groups was detected by qRT-PCR. Expression of miR-133a was significantly higher in the control group than in the observation group. ***there was a significant difference between the two groups (P<0.05).

Table 4. Relative expression of miR-133a and miR-206 in normal pregnancy subgroup and abnormal pregnancy subgroups

Group	miR-133a	miR-206
Normal pregnancy subgroup (n=55)	0.630	0.742
Abnormal pregnancy subgroup (n=34)	0.353	0.441
t value	12.160	12.655
P value	<0.001	<0.001

resistance, and genetic factors [22]. At present, the pathogenesis of PE has not been elucidated. In recent years, with advancements in molecular biology techniques, an increasing number of in-depth studies have focused on the pathogenesis and progression mechanisms of PE. Moreover, miRs are novel molecules that have been attracted much attention [23]. They are endogenous non-coding conserved small single-chain RNA molecules, with a high time-sequence and specificity [24]. Studies [25] have shown that miRs can specifically bind to target genes, participating in the development and progression of diseases. They are also involved in regulation of major processes, including cell proliferation, apoptosis, and growth, as well as development and progression of tumors.

miR-133a, a member of the miR-133 family (miR-133a and miR-133b), has been classified as miR-133a-1 and miR-133a-2. There is evidence [26] for low expression of miR-133a in multiple tumors, especially for its obvious inhibitory effects on cell migration and invasion in tumors. Qiu et al. [27] found that miR-133a expression was different in gastric cancer and that miR-133a can suppress cell proliferation, migration, invasion, and cell cycle in gastric cancer by targeting Sp1. Moreover, miR-206 plays key roles in tumors and cardiovascular diseases by regulating cell apoptosis, proliferation, and differentiation, as well as angiogenesis [28]. However, whether there is differential expression of miR-133a and miR-206 in PE patients has not been reported in previous studies. The current study evaluated expression of miR-133a and miR-206 in the peripheral blood of pregnant or maternal women with PE. In the control group, expression of miR-133a and miR-206 was significantly downregulated, compared to that in the observation group, suggesting that low expression of miR-133a and miR-206 may serve as a potential indicator of PE. This study investigated expres-

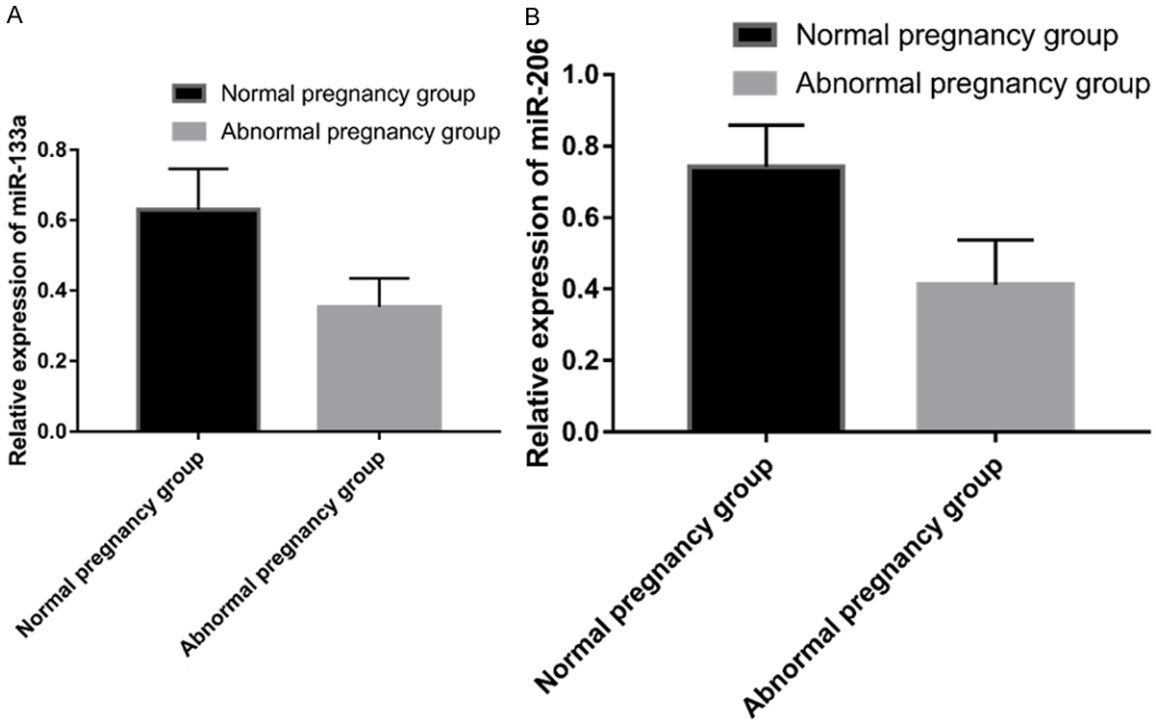

Figure 2. Relative expression of miR-133a and miR-206 in the peripheral blood of patients with abnormal pregnancy outcomes and normal pregnancy outcomes. A. qRT-PCR used to detect miR-133a expression in the peripheral blood of the two groups of patients, showing a significant difference in expression between patients in the abnormal pregnancy outcome group and those in the normal pregnancy outcome group. B. Expression of miR-206 in the peripheral blood of the two groups of patients was analyzed by qRT-PCR. There was a statistically significant difference in miR-206 expression between the abnormal pregnancy outcome group and normal pregnancy outcome group. There was a significant difference in miR-206 expression in the peripheral blood between patients with normal pregnancy outcomes and those with abnormal pregnancy outcomes. ***there was a significant difference between the two groups ($P<0.05$).

sion of miR-133a and miR-206 in the peripheral blood. Statistically significant differences were observed in comparing low expression of miR-133a and miR-206 between normal and abnormal pregnancy groups, suggesting that expression of miR-133a and miR-206 is closely correlated with pregnancy outcomes. Thus, risk factor analysis was conducted for patients in the observation group, finding that miR-133a and miR-206 are independent risk factors of abnormal pregnancy outcomes in PE patients. There is evidence [29, 30] that miR-133a can inhibit MAPK signaling pathways by targeted regulation of activity/expression levels of LIM and SH3 proteins. MAPK signaling pathways affect endothelial function, immunity, inflammation, and cell nourishment. They are even involved in PE pathogenesis. Thus, results suggest that miR-133a may affect MAPK signaling pathways via regulation of target genes, inducing differential expression in PE progression. Yue et al. [31] reported that miR-206 can regulate HIF-1α/Fhl-1 signaling pathways and that it is involved in hypoxia-induced pulmonary arterial hypertension. Furthermore, Jin et al. [32] detected expression of miR-206 in the serum of 82 patients with heart disease, reporting low expression of miR-206 in the serum. Based on the findings above, it was speculated that miR-206 may correlate with the maintenance of blood pressure in patients.

Correlation analysis was conducted for expression of miR-133a and miR-206 in the peripheral blood of patients in the two observation groups, finding a positive correlation between expression of miR-133a and miR-206. Thus, results suggest that miR-133a and miR-206 may share common upstream target genes. At present, several studies have focused on the roles of miRs in PE. In this study, expression of miR-133a and miR-206 was significantly decreased in the peripheral blood of PE patients, compared to that in healthy pregnant women.

Table 5. Univariate analysis of normal pregnancy and abnormal pregnancy [n (%)]

Factor	Normal pregnancy subgroup (n=55)	Abnormal pregnancy subgroup (n=34)	t value	*P* value
Age (age)	26.54	29.84	6.643	<0.001
Gestational age of delivery	36.26	36.78	1.386	0.169
BMI during delivery (kg/m^2)	28.38	28.97	0.963	0.338
MABP (mmHg)	121.21	143.94	13.260	<0.001
Neonatal weight (g)	3612.92	3616.65	0.034	0.973
Intraoperative bleeding volume (mL)	133.10±8.86	132.21	0.502	0.617
Urine protein (g/L)	2.48	4.52	13.478	<0.001
Smoking history			0.454	0.500
Yes	20 (36.36)	10 (29.41)		
No	35 (63.64)	24 (70.59)		
History of alcoholism			0.016	0.900
Yes	3 (5.26)	2 (5.88)		
No	54 (94.74)	32 (94.12)		
Degree of education			0.635	0.425
> Junior middle school	36 (65.45)	25 (73.53)		
≤5 (73.53) school	19 (34.55)	9 (26.47)		
Residence			0.375	0.540
Village	16 (29.09)	12 (35.29)		
City	39 (70.91)	22 (64.71)		

Table 6. Assignment table

Factor	Assignment
Age	<26 years old =1, ≥26 years old =0
MABP	A continuous variable which is analyzed using raw data
Urine protein	A continuous variable which is analyzed using raw data
miR-133a	A continuous variable which is analyzed using raw data
miR-206	A continuous variable which is analyzed using raw data

Table 7. Multivariate logistic regression analysis

Factor	β	SE	X^2	P	OR	95% CI
Age	0.008	0.014	0.335	0.563	1.009	0.971-1.015
MABP	0.005	0.008	0.352	0.549	1.005	0.993-1.028
Urine protein	0.023	0.054	0.178	0.673	1.029	0.929-1.145
miR-133a	0.187	0.065	9.265	0.002	1.204	1.058-1.362
miR-206	0.098	0.032	8.822	0.001	1.096	1.029-1.155

Patients with abnormal pregnancy outcomes showed a more significant decrease than those with normal pregnancy outcomes. Risk factor analysis showed that miR-133a and miR-206 may be independent risk factors of abnormal pregnancy outcomes in PE patients, suggesting that miR-133a and miR-206 may serve as potential observation indices for PE patients with abnormal pregnancy outcomes.

However, there were some limitations of this study. First, the samples were isolated from the peripheral blood by minimal invasion, but expression of miR-133a and miR-206 was not detected in the placentas of pregnant or maternal women. Second, due to the lack of an in-depth investigation into relevant mechanisms, this study was not able to elucidate the regulatory roles of miR-133a and miR-206. Therefore, present conclusions should be verified by future studies with large sample sizes, *in vitro* experiments, and animal experiments.

In conclusion, expression levels of miR-133a and miR-206 are downregulated in the peripheral blood of PE patients, possibly correlating with poor pregnancy outcomes.

Acknowledgements

This work was supported by the Nature Science Foundation Project of Gansu Province (Grant

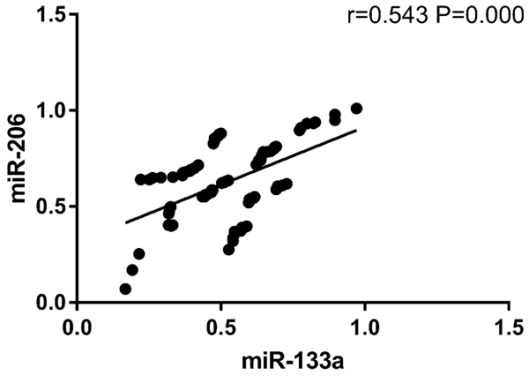

Figure 3. Correlation between miR-133a and miR-206 expression in the peripheral blood of patients in the observation group, analyzed by Pearson's correlation analysis. A positive correlation between expression of miR-133a and miR-206 in the peripheral blood of the observation group was noted. (r=0.543, P≤0.001).

number: 150RJZA280); Health-industry Scientific Research Project of Gansu Province (Grant number: GWGL2014-33) and Key Basic Scientific Research Projects in Central Colleges and Universities (Grant number: 31920190098).

Disclosure of conflict of interest

None.

Address correspondence to: Rong Ma, Medical College, Northwest Minzu University, No.1, Northwest New Village, Lanzhou 730030, Gansu, China. Tel: +86-0931-2938150; E-mail: rongmee@163.com

References

[1] Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M and Verlohren S. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med 2016; 374: 13-22.

[2] Chen Q, Sousa JD, Snowise S, Chamley L and Stone P. Reduction in the severity of early onset severe preeclampsia during gestation may be associated with changes in endothelial cell activation: a pathological case report. Hypertens Pregnancy 2016; 35: 32-41.

[3] Nakanishi S, Aoki S, Nagashima A and Seki K. Incidence and pregnancy outcomes of superimposed preeclampsia with or without proteinuria among women with chronic hypertension. Pregnancy Hypertens 2017; 7: 39-43.

[4] Amaral LM, Cornelius DC, Harmon A, Moseley J, Martin JN and LaMarca B. 17-Hydroxyprogesterone caproate significantly improves clinical characteristics of preeclampsia in the reduced uterine perfusion pressure rat model. Hypertension 2015; 65: 225-231.

[5] Hummers LK. Systemic vasospasm. New York: Springer; 2015.

[6] Zhou M. Tei index in preeclampsia pregnant women and its correlation with the degree of endothelial injury and hypertension. Journal of Hainan Medical University 2017; 23.

[7] Seely EW, Tsigas E and Rich-Edwards JW. Preeclampsia and future cardiovascular disease in women: how good are the data and how can we manage our patients? Semin Perinatol 2015; 39: 276-283.

[8] Rupaimoole R and Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203-222.

[9] Chang TC and Mendell JT. High-throughput characterization of primary microRNA transcripts. Methods Mol Biol 2018; 1823: 1-9.

[10] A B, M C and T H. Single nucleotide variations within and around microRNA-binding sites. 2018.

[11] Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, Draghici S, Espinoza J, Kusanovic JP, Mittal P, Hassan SS and Kim CJ. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol 2007; 196: 261, e1-e6.

[12] M ML, A RD and M PS. Energy restriction upregulates circulating myomiR expression in vivo and in vitro. FASEB J 2017; 31: 311-316.

[13] Davison JM and Lindheimer MD. ISSHP, an association saving the lives of pregnant women and their babies: (the international society for the study of hypertensions in pregnancy (ISSHP): its history). Pregnancy Hypertens 2017; 7: 2-28.

[14] Shabtaie SA, Gerkowicz SA, Kohn TP and Ramasamy R. Role of abnormal sperm morphology in predicting pregnancy outcomes. Curr Urol Rep 2016; 17: 67.

[15] Kanasaki K. Glucose intolerance and insulin resistance: relevance in preeclampsia. 2018.

[16] Joensuu H, Kellokumpu-Lehtinen PL, Huovinen R, Jukkola-Vuorinen A, Tanner M, Kokko R, Ahlgren J, Auvinen P, Lahdenperä O and Kosonen S. Adjuvant capecitabine in combination with docetaxel, epirubicin, and cyclophosphamide for early breast cancer: the randomized clinical FinXX trial. JAMA Oncol 2017; 3: 793-800.

[17] van Esch JJA, van Heijst AF, de Haan AFJ and van der Heijden OWH. Early-onset preeclampsia is associated with perinatal mortality and severe neonatal morbidity. J Matern Fetal Neonatal Med 2017; 30: 2789-2794.

[18] Kusuma GD, Abumaree MH, Perkins AV, Brennecke SP and Kalionis B. Reduced aldehyde

dehydrogenase expression in preeclamptic decidual mesenchymal stem/stromal cells is restored by aldehyde dehydrogenase agonists. Sci Rep 2017; 7: 42397.
[19] Jim B and Karumanchi SA. Preeclampsia: pathogenesis, prevention, and long-term complications. Semin Nephrol 2017; 37: 386-397.
[20] Bahr BL, Price MD, Merrill D, Mejia C, Call L, Bearss D and Arroyo J. Different expression of placental pyruvate kinase in normal, preeclamptic and intrauterine growth restriction pregnancies. Placenta 2014; 35: 883-890.
[21] Rani A, Chavan-Gautam P, Mehendale S, Wagh G and Joshi S. Differential regional fatty acid distribution in normotensive and preeclampsia placenta. BBA Clin 2015; 4: 21-26.
[22] Govindsamy A, Naidoo S and Cerf ME. Cardiac development and transcription factors: insulin signalling, insulin resistance, and intrauterine nutritional programming of cardiovascular disease. J Nutr Metab 2018; 2018: 8547976.
[23] Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, Huang WC, Sun TH, Tu SJ, Lee WH, Chiew MY, Tai CS, Wei TY, Tsai TR, Huang HT, Wang CY, Wu HY, Ho SY, Chen PR, Chuang CH, Hsieh PJ, Wu YS, Chen WL, Li MJ, Wu YC, Huang XY, Ng FL, Buddhakosai W, Huang PC, Lan KC, Huang CY, Weng SL, Cheng YN, Liang C, Hsu WL and Huang HD. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. Nucleic Acids Res 2018; 46: D296-D302.
[24] Ha M and Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.
[25] Agarwal V, Bell GW, Nam JW and Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife 2015; 4.
[26] Elshimy R, A El-Mahdy H, A Mansour O, Badr M, M Ali A and M Ali Mir A. MiR-133a and MiR-155 as potential minimally invasive biomarkers in breast cancer. 2017.
[27] Qiu T, Zhou X, Wang J, Du Y, Xu J, Huang Z, Zhu W, Shu Y and Liu P. MiR-145, miR-133a and miR-133b inhibit proliferation, migration, invasion and cell cycle progression via targeting transcription factor Sp1 in gastric cancer. FEBS Lett 2014; 588: 1168-1177.
[28] Zhou J, Shao G, Chen X, Yang X, Huang X, Peng P, Ba Y, Zhang L, Jehangir T, Bu S, Liu N and Lian J. miRNA 206 and miRNA 574-5p are highly expression in coronary artery disease. Biosci Rep 2015; 36: e00295.
[29] Wang H, An H, Wang B, Liao Q, Li W, Jin X, Cui S, Zhang Y, Ding Y and Zhao L. miR-133a represses tumour growth and metastasis in colorectal cancer by targeting LIM and SH3 protein 1 and inhibiting the MAPK pathway. Eur J Cancer 2013; 49: 3924-3935.
[30] D'Oria R, Laviola L, Giorgino F, Unfer V, Bettocchi S and Scioscia M. PKB/Akt and MAPK/ERK phosphorylation is highly induced by inositols: novel potential insights in endothelial dysfunction in preeclampsia. Pregnancy Hypertens 2017; 10: 107-112.
[31] Yue J, Guan J, Wang X, Zhang L, Yang Z, Ao Q, Deng Y, Zhu P and Wang G. MicroRNA-206 is involved in hypoxia-induced pulmonary hypertension through targeting of the HIF-1alpha/Fhl-1 pathway. Lab Invest 2013; 93: 748-759.
[32] Jin P, Gu W, Lai Y, Zheng W, Zhou Q and Wu X. The circulating MicroRNA-206 level predicts the severity of pulmonary hypertension in patients with left heart diseases. Cell Physiol Biochem 2017; 41: 2150-2160.