

## Original Article

# Correlation of miRNA and VEGF expression with the outcome of early-onset severe preeclampsia in patients receiving expectant treatment

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**Abstract:** The present study was planned to explore the relationship between miRNA and VEGF expression in serum as well as placenta tissue with the outcome of early-onset severe preeclampsia (EOSP) in patients receiving expectant treatment. Sixty EOSP patients who had expectant treatment indications were divided into the success group (n = 46) and the failure group (n = 14) according to the pregnancy outcomes. miR-210 and miR-155 expression levels were studied in serum, ante partum, and in placenta tissue. The vascular endothelial growth factor (VEGF) and soluble VEGF receptor 1 (sFlt-1) expression levels were also explored. miR-210 and miR-155 expression levels in serum and placenta tissue before treatment, ante partum, and after accouchement of the success group were significantly lower than those of the failure group. Further, VEGF expression levels in serum and placenta tissue before treatment, ante partum, and after accouchement of the success group were significantly higher than those of the failure group. However, sFlt-1 expression levels in the success group showed a decrease in comparison to the failure group. The increase of miR-210, miR-155 levels, sFlt-1 levels, and the decrease of VEGF levels in EOSP patients might be correlated with the failure of expectant treatment.

**Keywords:** Early-onset severe preeclampsia (EOSP), expectant treatment, miRNAs, vascular endothelial growth factor (VEGF), soluble VEGF receptor 1 (sFlt-1)

## Introduction

Preeclampsia, which is a specific disease during the gestational period has an overall incidence of about 5~10%. It has been reported to be an important disease that could lead to mortality in both pregnant women as well as the fetus [1]. Early-onset severe preeclampsia (EOSP) is usually performed within 20 weeks ~ 32 or 34 weeks of pregnancy. It is often accompanied by severe hypertension, proteinuria, multiple organ failure, etc. The earlier the onset of the gestational week, the worse the outcome of pregnancy [2]. The development of obstetric and neonatal intensive care technology has allowed the application indications of expectant treatment. This significantly helped in the reduction of the perinatal mortality and ameliorated the long-term clinical outcomes of mother as well as infant [3]. The pathogenesis of EOSP might involve vascular endothelial injury, inflam-

mation, oxidative stress reaction, thrombosis, immune disorder, rennin-angiotensin metabolic disorder, etc. [4]. However, microvascular endothelial damage in placenta tissue is crucial, as it has been reported to play a key role in the occurrence of EOSP [5]. Vascular endothelial growth factor (VEGF) and its receptors are expressed in maternal serum as well as placental tissue throughout the pregnancy [6]. Moreover, VEGF, strongly promotes angiogenesis, cell proliferation, migration, invasion, and is of great significance in the physiological process of development of normal embryo to normal fetal development [7]. A study in the recent past [8] confirmed that VEGF expression in the peripheral circulation of patients with preeclampsia is abnormally decreased and is closely related to the severity of the disease.

Micro RNAs (miRNAs) could regulate the transcription of target genes and the functional

expression of target protein in eukaryotic organisms by the application of gene chip screening technology. Furthermore, it was noticed that [9] up-regulation or down-regulation of expression of multiple miRNAs affected the expression and activation of related mRNA, cytokine, and signaling pathways. This study aimed to study the correlation between miR-210, miR-155, and VEGF expression with the outcome of EOSP patients receiving expectant treatment.

### Materials and methods

#### *Study subjects*

Sixty patients, who were diagnosed as EOSP in our hospital from October 2015 to October 2016, were selected as study subjects. All fetuses were single live birth. Inclusion criteria: a) the patients had the disease that conformed to the diagnosis and classification criteria of the American Hypertension Education Program and had expectant treatment indications; b) the patients had better treatment compliance and perfect clinical data and signed the informed consent. Exclusion criteria: a) the patients were complicated with other diseases of pregnancy such as diabetes mellitus or had serious complications such as heart failure and so on, and the fetus suffered from dysplasia, such as deformity, severe hypoxia, etc.; c) the patients had other factors that affected the detection index, such as drug, autoimmune disease, etc.

#### *Study design*

The patients were divided into the success group (n = 46) and the failure group (n = 14) according to the pregnancy outcomes. In the success group, patients aged 22~30 years old, with an average of (25.5±3.6), and the gestational week onset was at 25~33 weeks (at average of 28.5±4.7 weeks). There were 39 primiparas and 7 pluriparas; the body mass index (BMI) was 22.5~24.6 kg/m<sup>2</sup>, with an average of (22.9±2.2) kg/m<sup>2</sup>, mean systolic pressure was 165~182 mmHg, with an average of (171.2±5.6) mmHg, mean diastolic pressure was 113~136 mmHg, with an average of (121.6±8.7) mmHg, mean leukuresis was 2.5~3.9 g/d, with an average of (2.8±0.5) g/d, and mean serum creatinine was 152.6~324.7 μmol/L, with an average of (185.9±36.6) μmol/L. In the failure group, patients aged 20~33 years old, with an aver-

age of (24.6±3.8), and the gestational week onset was at 24~32 weeks (at average of 27.7±4.8 weeks); there were 10 primiparas and 4 pluriparas; the body mass index (BMI) was 22.3~24.4 kg/m<sup>2</sup>, with an average of (22.5±2.7) kg/m<sup>2</sup>, mean systolic pressure was 169~187 mmHg, with an average of (175.5±5.9) mmHg, mean diastolic pressure was 114~138 mmHg, with an average of (122.7±8.5) mmHg, mean leukuresis was 2.4~3.8 g/d, with an average of (2.7±0.6) g/d, and mean serum creatinine was 146.9~315.7 μmol/L, with an average of (182.2±41.2) μmol/L. Baseline data of the two groups were comparable.

#### *Research methods*

The standard expectant treatment was utilized that included resting for sedation, eliminating pains, decreasing blood pressure, protecting liver and kidney, relieving spasm, expanding blood volume, diuresis, anticoagulation, correcting complications, etc. The termination of the pregnancy was dependent on the circumstances during treatment. Dexamethasone was used to promote fetal lung maturation with the dosage of 5 mg by intramuscular injection once every 12 h for 4 times. The mother and infant vital signs and fetal intrauterine growth were closely monitored. Criteria for judging the outcome of pregnancy: it was safe till 34 weeks of gestation; there were no serious complications in mother and infant and no dysplasia in the infant, which were regarded as the success. Otherwise, it meant failure.

The miR-210 and miR-155 expression levels in peripheral venous serum before treatment, ante partum, and in cord blood after accouchement as well as in placenta tissue after accouchement were detected by RT-PCR method. The main steps of RT-PCR method included extracting total RNA in cells by using routine Trizol agents, detecting concentration and purity by UV spectrophotometer, and synthesizing cDNA by reverse transcription kit. The primer sequences were synthesized by Sangon Biotech (Shanghai) Co., Ltd., according to Gene Band sequences, namely miR-210: (F) 5'-GGTTTCATCCAGGATCGAGCAGG-3', (R) 5'-ACAAAGATGGTCACGGTCTGCC-3', 445bp; miR-155: (F) 5'-ACTACTTCTCCCGCCGCTAC-3', (R) 5'-GAAATCAAACAGAGGCCGCATG-3', 332bp; GAPDH (F): 5'-CGCGAGAAGATGACCCAGAT-3', (R): 5'-GCACTGTGTTGGCGTACAGG-3', 225 bp. The reaction scheme

## miRNAs VEGF and EOSP receiving expectant treatment

**Table 1.** Expression levels of miR-210 and miR-155

Groups		Success group	Failure group	t	p
miR-210	Before treatment	0.2647±0.0768	0.3562±0.0968	6.637	0.000
	Ante partum	0.2347±0.0824	0.4242±0.1263	7.859	0.000
	After accouchement	0.2185±0.0659	0.4056±0.1147	7.654	0.000
	Placenta tissue	0.3659±0.1021	0.5263±0.1427	15.326	0.000
miR-155	Before treatment	0.2832±0.1023	0.3965±0.1212	7.625	0.000
	Ante partum	0.2645±0.1241	0.4574±0.1635	8.429	0.000
	After accouchement	0.2495±0.1162	0.4326±0.1524	8.325	0.000
	Placenta tissue	0.3758±0.1527	0.5642±0.2121	16.325	0.000

**Table 2.** Expression levels of VEGF and sFlt-1 (µmol/L)

Groups		Success group	Failure group	t	p
VEGF	Before treatment	264.9±102.3	175.9±96.7	8.625	0.000
	Ante partum	334.7±132.6	165.4±85.8	10.324	0.000
	After accouchement	312.2±142.5	135.8±76.6	9.623	0.000
	Placenta tissue	356.5±126.9	247.8±103.5	18.527	0.000
sFlt-1	Before treatment	165.9±78.8	202.3±93.6	10.322	0.000
	Ante partum	154.7±65.9	236.9±82.3	15.654	0.000
	After accouchement	136.9±58.8	214.8±76.6	13.527	0.000
	Placenta tissue	202.3±95.7	254.7±65.5	21.232	0.000

was 2 µl cDNA+3 µl of upstream and downstream primers +0.5 µl Taq polymerase +1 µl dNTPs+3 µl MgCl<sub>2</sub>+5 µl 10× Buffer +2.5 µl ddH<sub>2</sub>O<sub>2</sub>. The reaction conditions were 95°C for 5 min, 95°C for 30 s, 58°C for 30 s and 72°C for 60 s, a total of 30 cycles, and 72°C for 10 min for the end. PCR products were identified by 2% agarose gel electrophoresis, ultraviolet spectral imaging was analyzed by a gel imaging analysis system, the digital camera was used for capturing of photographs and the gray values were analyzed. The results were expressed by 2<sup>-ΔΔCt</sup> method. ELISA reagents were purchased from Jiangsu Beyotime Biotechnology Co., Ltd. Microplate Reader was purchased from Bio-Rad company (Hercules, California, USA). The operation was strictly conducted in accordance with the instructions.

### Statistical methods

SPSS 20.0 software was adopted for statistical analyses. The measurement data are expressed as mean ± standard deviation, and independent sample t test used for the comparison between groups. Moreover, ANOVA followed by *post hoc* least significant difference (LSD) was also performed for intergroup comparisons. The enumeration data were expressed by case

or percentage, and Chi square test was used for the comparison between groups. *P* < 0.05 suggested that the difference was statistically significant.

### Results

#### Expression levels of miR-210 and miR-155

The miR-210 and miR-155 expression levels in serum and placenta tissue before treatment, ante partum, and after accouchement of the success group were significantly lower in comparison to the failure group (*P* < 0.05) (**Table 1**).

#### Expression levels of

#### VEGF and sFlt-1

The VEGF expression levels in serum and placenta tissue before treatment, ante partum, and after accouchement of the success group were significantly higher than those of the failure group. On the other hand, sFlt-1 expression levels in the success group were significantly lower in comparison to the failure group (*P* < 0.05) (**Table 2**).

### Discussion

It has been reported that the degree of organ injury in EOSP does not parallel with blood pressure and leukuresis levels but the target organ gets seriously damaged [10]. Neonatal survival rate is often increased with the delay of gestational week of the attack. On the contrary, the fetus hardly survives if the pregnant woman suffers from EOSP at 24 weeks of gestation [11]. In an earlier study, Withagen et al. [12] enrolled 574 patients with pregnancy less than 32 weeks and were receiving expectant treatment. This study confirmed that the complications of mother and infant who continuously received expectant treatment for 2 weeks were minimal. Moreover, the follow-up of children until 4~12 years old showed that the incidence

rate of various acute and chronic respiratory diseases were also minimal. The optimal duration of expectant treatment remains to be explored and there is no uniform understanding. Cesarean section has become the method of choice for the termination of pregnancy. It is of a great significance to reduce the deterioration of the condition as it allows accurate grasp of the delivery time and ameliorates the fetal hypoxia environment [13].

The clinical outcome of EOSP is often poor, so the early diagnosis and prognosis evaluation have important values in improving the success of disease treatment. This study showed that miR-210 and miR-155 expression levels in serum and placenta tissue of the success group were significantly lower than those of the failure group at different time points. Also, the VEGF expression levels were significantly elevated. On the other hand, the sFlt-1 expression levels were decreased. This confirmed that the increase of miR-210, miR-155 expression levels, the decrease of VEGF expression levels, and the increase of sFlt-1 expression levels in serum as well as placenta tissue of EOSP patients might be correlated with the failure of expectant treatment. Therefore, early detection is of great clinical significance in predicting the outcome of pregnancy. Earlier studies have confirmed that [14-17] the increased expression of miR-210 and miR-155 are closely associated with the occurrence of pregnancy-induced hypertension, preeclampsia, and eclampsia. It often has a negative regulatory effect on target genes. Moreover, 20 target genes have been screened by using mRNA expression profile high-throughput microarray chip, such as VEGF and hypoxia inducible factor-1 (HIF-1) [18, 19].

VEGF participates in the processes of nitric oxide (NO), endothelin-1 (ET-1), tissue plasminogen and other active substances that are released by endothelial cells. In this way it helps in the regulation of vascular contractile response and the disturbance of blood coagulation [20]. Another study considers that a [21] "toxic factor" in the placenta and circulation of preeclampsia patients is an important factor that could cause the injury of endothelial cells. sFlt-1 is the splice variant of VEGF receptor Flt-1. Multiple studies indicated that [22, 23] in patients with preeclampsia, serum levels of sFlt-1 were significantly increased within 6~11 weeks before clinical symptoms appeared, and

recovered to the basic levels at 48 h after childbirth. Maynard et al. [24] found that the pregnant rats who were injected with excessive sFlt-1 would suffer from hypertension, proteinuria, glomerular endothelial hyperplasia, and other similar preeclampsia symptoms. On the other hand, exogenous injection of VEGF could alleviate the symptoms, suggesting that abnormal increases of sFlt-1 levels could inhibit VEGF from regulating the normal physiological function of endothelial cells.

The significance of this study is to indicate that miR-210, miR-155, VEGF, and sFlt-1 play similarly important roles in the pregnancy outcome of EOSP receiving expectant treatment. The deficiency in the present study is the lack of negative controls, namely EOSP patients without receiving expectant treatment and normal controls, namely healthy pregnant women without pregnancy complications. Therefore, the present study was not able to confirm the above indicators are the only ones involved in the occurrence and development of the disease. Further, future studies are required for confirmation of these factors as targets for early intervention.

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### Disclosure of conflict of interest

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