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Original Article

The predictive value of microRNA in early hypertensive disorder complicating pregnancy (HDCP)

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Abstract: Objective: To examine the predictive value of microRNA (miRNA) in hypertensive disorder complicating pregnancy (HDCP). Methods: 102 pregnant women with HDCP admitted to our hospital from March 2017 to June 2019 were recruited as the study cohort and randomly divided into an HDCP group, a mild preeclampsia group, and a severe preeclampsia group, with 34 patients in each group. In addition, 34 healthy pregnant women who underwent pregnancy tests in our hospital were recruited as the normal group. The relative expressions of plasma miR-19a, miR-126, and miRNA-210 in were measured. A Pearson correlation analysis was used to analyze the correlations between the miR-19a, miR-181b, and miRNA-210 expressions and the severity of HDCP. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic efficacy of the miR-19a, miR-126, and miRNA-210 expressions. Results: The miR-19a and miRNA-210 expressions were higher in the HDCP group, the mild preeclampsia group, and the severe preeclampsia group than they were in the normal group, and the miR-126 expression was lower (all $P<0.05$). The miR-19a, miR-126, and miRNA-210 expressions were different among the four groups ($P<0.05$). The miR-19a and miRNA-210 expression levels in the severe preeclampsia group were higher than they were in the HDCP group, and the miR-126 expression was lower ($P<0.05$). A Pearson correlation analysis showed the miR-19a and miR-210 levels in the HDCP patients were positively correlated with the severity of the disease ($P<0.05$), and the miR-126 level is negatively correlated with disease severity ($P<0.05$). Our ROC curve analysis demonstrated that the miR-19a, miR-126, and miR-210 levels have a predictive value for HDCP. The areas under the curve were 0.800, 0.633, and 0.723 , the sensitivities were 81.2%, 71.4%, and 80.2% , and the specificities were 73.5%, 67.5%, 81.5%. Additionally, the area under the curve of the combination of the three was 0.896, and the sensitivity and specificity were 90.5% and 93.9% respectively. Conclusion: miR-19a, miR-126, and miR-210 are strongly connected to the severity of HDCP and can be used as a sensitive indicator to predict HDCP patients clinically.

Keywords: MicroRNA, hypertension during pregnancy, pre-eclampsia

Introduction

Hypertensive disorder complicating pregnancy (HDCP) is a series of diseases with pregnancy and high blood pressure involved, and is one of the leading causes of illness and death among mothers and babies [1]. The primary clinical manifestations of the diseases include elevated blood pressure, edema, and systematic multiple organ damage. In severe cases, coma and convulsions may even occur, and they have a negative impact on the health of mothers and babies [2]. According to reports [3], the incidence rate of this disease in China is 9.4%, and it is approximately 7%-12% abroad. The pathogenesis of HDCP remains obscure, so an early diagnosis and assessment of the severity of the disease is therefore a vital way to implement effective treatment to delay the progression of HDCP. Multiple recent studies have found that [4] miRNAs are involved in regulating apoptosis, proliferation, cell differentiation, organ formation, and the aging of organisms and are closely associated with the occurrence of diseases such as inflammation, diabetes, malignant tumors, and hypertension. Previous studies reported [5, 6] that, among a variety of microRNAs, miR-19a, miRNA-210, and miR-126 are highly expressed in placental tissue and the peripheral blood of pregnant women with preeclampsia and are strongly connected to preeclampsia. The disease's occurrence and development are closely related. Nevertheless, there is paucity of evidence regarding microR-

Table 1. Comparison of the baseline data between the two groups

Group	Case group (n=102)	Control group (n=34)	t	P
Age (years)	27.26±5.12	27.30±5.10	0.039	0.969
gestational weeks (weeks)	29.40±6.48	28.77±6.45	0.492	0.624
Gravidity (times)	1.26±0.26	1.30±0.24	0.791	0.430
BIM (Kg/m^2)	23.14±3.12	23.26±3.17	0.194	0.847

NA and HDCP, and the differential expressions of microRNA in the plasma of HDCP and normal pregnant women are also different from those of non-pregnant hypertension. The current studies hence attempted to measure and compare the differential expressions of microRNAs in HDCP and to generate evidence that identifies new and non-invasive diagnostic indicators for HDCP.

Materials and methods

Subjects

This study was a controlled analysis conducted on 102 pregnant women with HDCP admitted to our hospital from March 2017 to June 2019, and these participants were assigned into the case groups. Additionally, 34 healthy pregnant women who underwent pregnancy screening in our hospital during the same period were placed in the normal group. The *P*-value for the general comparison was greater than 0.05, and this suggested the fitness of the participants (**Table 1**). According to the HDCP diagnosis and classification criteria, the case group patients were classified into 34 patients in the HDCP group, 34 patients in the mild preeclampsia group, and 34 patients in the severe preeclampsia group. This study was registered and approved by the medical ethics committee of our hospital, and the patients and their families involved in the study participated voluntarily.

Inclusion criteria: (1) Patients who met the diagnostic criteria for hypertension during pregnancy [7]. (2) Patients with a gestational age ≥20 weeks. (3) Patients with a systolic blood pressure ≥140 mmHg (1 mmHg=0.133 kPa) and a diastolic blood pressure ≥90 mmHg and whose blood pressure returned to normal within 12 weeks after delivery and whose urine protein tested negative. Exclusion criteria: (1) Patients who used immunosuppressants and glucocorticoid hormones for a long time. (2) Patients suffering from mental illnesses, cancer, or systemic inflammation diseases. (3) Patients with coagulation dysfunction. (4) Patients with cardiopulmonary insufficiency.

Methods

Fasting venous blood in the early morning was obtained from all the pregnant women, centrifuged to procure RNA precipitation, mixed with absolute ethanol, centrifuged and filtered, and then washed. Then miRNA with higher genome quality was extracted, and miR-19a (5'-UGUGCAAAUCUAUGCAAAACUGA-3'), miR-126 (5'-UCGUACCGUGAGUAAUAAUGCG-3') and miR-210 (5'-AGCCCCUGCCCACCGCACACUG-3') were taken as the target genes. A biospectrophotometer was used to determine the OD value of the sample to evaluate the purity of the RNA to ensure that it was not adulterated with phenol or protein. Reverse transcription was carried out with hsa-miR-19a, miR-126, and miRNA-210 reverse transcription primers, the internal reference gene was U6, and the fluorescence quantitative PCR was used for the amplification. The relative miR-19a, miR-126, and miRNA-210 expression levels were calculated using the 2-ΔΔct method.

Statistical methods

This study was carried out using the SPSS 22.0 statistical package. [n (%)] and x ± s were calculated for the qualitative data and quantitative data, respectively, and chi-square and t-tests were respectively used to determine whether the results differed statistically. Correlation was analyzed using Pearson correlation analyses, and ROC curves were calculated to evaluate the diagnostic value of miR-19a, miR-126, and miRNA-210. Significance in the current study was claimed at a *P*-value of <0.05.

Results

The serum miR-19a, miR-126, and miRNA-210 expression levels

The serum miR-19a and miRNA-210 levels were found to be higher in the case group as compared with the control group; in contrast, the miR-126 level was shown to be lower when compared with the control group, and the difference between the two groups was significant (P<0.05, **Table 2**).

Table 2. Comparison of the serum miR-19a, miR-126, and miRNA-210 expression levels in the two groups of patients

Group	miR-19a	miR-126	miRNA-210
Case group (n=102)	9.65±3.78	1.98±0.66	6.77±2.43
Control group (n=34)	2.02±0.78	8.95±3.54	1.50±0.54
t	11.530	11.290	12.340
P	<0.001	<0.001	<0.001

Table 3. Comparison of the miR-19a, miR-181b, and miRNA-210 expression levels in each HDCP subgroup

Group	miR-19a	miR-126	miRNA-210
HDCP group (n=34)	6.60±3.78	15.65±5.23	3.37±1.41
mild preeclampsia group (n=34)	11.64±3.80*	9.38±2.57*	6.63±2.04*
severe preeclampsia group (n=34)	17.88±5.72**	5.95±2.56**	10.65±4.33**
t	5.483	2.873	1.678
P	<0.001	<0.001	0.098

Note: *means compared with the HDCP group, P<0.05; **means compared with the mild preeclampsia group, (P<0.05).

Table 4. The correlation between the miR-19a, miR-181b, and miRNA-210 expression levels in the plasma of the HDCP pregnant women and the severity of the disease

Item	miR-19a		miR-126		miRNA-210	
	r	*P*	*r*	*P*	*r*	*P*
Severity of the disease	0.336	<0.001	-0.146	0.002	0.256	0.014

The serum miR-19a, miR-181b, and miRNA-210 expression levels in the patients with different degrees of HDCP

The differences in the serum miR-19a, miR-126, and miRNA-210 levels in the HDCP patients with different degrees of disease were statistically significant (P<0.05). Higher miR-19a and miRNA-210 expressions were identified in the severe preeclampsia group compared with the HDCP group, and the miR-126 expressions were found to be lower when compared with the HDCP group (P<0.05). See **Table 3**.

The correlation between the miR-19a, miR-181b, and miRNA-210 expressions in the plasma of the pregnant women with HDCP and the disease severity

Our Pearson correlation analysis showed that the serum miR-19a and miR-210 expressions in the HDCP patients were positively correlated with the severity of the disease (P<0.05) and miR-126 was negatively associated with the severity of the disease (P< 0.05), as presented in **Table 4**.

The diagnostic value of serum miR-19a, miR-126, miR-210 in HDCP

The findings of our ROC curve analysis demonstrated that the serum miR-19a, miR-126, and miR-210 expressions have a certain predictive value for HDCP. The areas under the curve were 0.800, 0.633, and 0.723, the sensitivities were 81.2%, 71.4%, and 80.2%, and the specificities were 73.5%, 67.5%, 81.5%. Additionally, the area under the curve of the combination of the three was 0.896, and the sensitivity and specificity were 90.5% and 93.9%. The comparison of the areas under the curve between the three indicators was insignificant (P>0.05), see **Table 5** and **Figure 1**.

Discussion

MicroRNA is a nucleic acid substance composed of about 80 single-stranded small molecules [8]. Previous studies have shown [9, 10] that up to 1/3 of human genes are potential target genes of microRNAs, which can inhibit target gene expression by binding to target gene translations and participating in the occurrence and regulation of diseases. There are many types of miRNA, and most of them have the sequential and phase characteristics of differentiation, but the biological functions of the different types are also very discernable, so they have a greater impact on gene expression, and even play direct regulatory roles [11]. According to earlier studies [12], MiRNA can be viewed as a biomarker of hypertension and has a high diagnostic value. Accordingly, numerous trials have screened various types of microR-

Table 5. The diagnostic value of the serum miR-19a, miR-126, and miR-210 expression levels in HDCP

Indicators	Cut-off value	AUC	95% CI	Sensitivity	Specificity
miR-19a	9.435	0.800	0.721~0.945	81.2%	73.5%
miR-126	8.642	0.633	0.601~0.705	77.4%	87.5%
miR-210	5.150	0.723	0.638~0.876	80.2%	81.5%
Combined levels	-	0.896	0.802~0.945	90.5%	93.9%

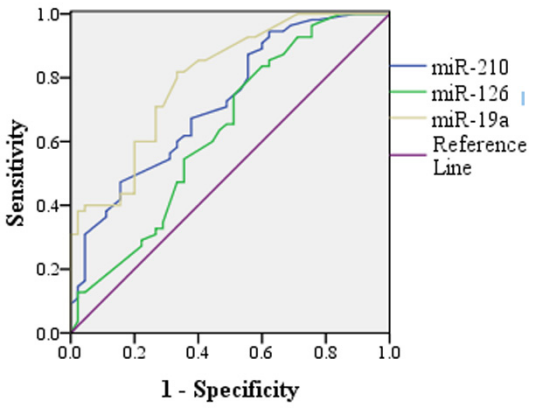

Figure 1. An ROC curve analysis of the diagnostic value of serum miR-19a, miR-126, and miR-210 in HDCP.

NA, attempting to find more accurate markers in an effort to improve the reliability of their hypertension biomarkers. A trial comparing placental miRNAs in the early and late stages of pregnancy using chip detection reported [13, 14] that the miRNAs that promote angiogenesis and express anti-apoptotic properties in the placenta of early pregnancy are dominantly expressed, but those that promote cell differentiation in the placenta of late pregnancy are strongly expressed, so it was suggested that miRNA plays an important role in placental development. Nevertheless, few studies have been conducted on microRNA and HDCP. Due to the differential expressions of microRNA in the plasma of pregnant women with HDCP and normal pregnant women, this study aimed to test the differential expressions of serum microRNA in HDCP patients.

HDCP can cause damage to a variety of tissues and organs of the body, and can seriously threaten the lives of mothers and perinatal babies. During pregnancy, the microRNA levels in placental tissue are high, among which miR-19a accumulates in a large amount, and clinical studies have confirmed that the expression levels in HDCP increase significantly [15]. miRNA-126 is highly expressed specifically in the endothelial cells, and it is a miRNA closely related to angiogenesis and plays a role in maintaining the integrity of blood vessels and the homeostasis of endothelial cells [16]. A high expression of miR-210 in pregnant women can lead to abnormal trophoblast cell function, which can result in an insufficient blood supply to the placenta. In preeclampsia placental trophoblast cells however, the expression of miR-210 is up-regulated and can regulate cell proliferation, differentiation, and hypoxia [17]. Studies have shown [18, 19] that the elevated expression of miR-210 under hypoxia can be used to screen for the risk of preeclampsia in pregnant women. It is involved in the pathogenesis of a variety of pregnancy-related diseases and can serve as a potential biomarker. Therefore, we selected miR-19a, miR-126, and miR-210 as the target genes in this study, and the plasma miR-19a, miR-126, and miR-210 expression levels in the HDCP and control groups were compared and analyzed. We found that higher miR-19a and microRNA-210 expression levels were observed in the HDCP group compared with the control group, and the miR-126 expression was lower than it was in the control group ($P<0.05$). Our findings indicate that miR-19a, miR-126, and miR-210 were significantly different in the HDCP and control groups, and this may provide a basis for viewing miR-19a, miR-126, and miR-210 as biomarkers for HDCP.

An early clinical diagnosis and timely effective treatment and preventive measures for HDCP are particularly important for improving pregnancy outcomes because HDCP causes many complications. Therefore, for HDCP, the determination of peripheral plasma biomarkers is of great significance. To further clarify the relationship between the expressions of miR-19a, miR-126, miR-210, and HDCP, the authors divided the case group into an HDCP group, a

mild preeclampsia group, and a severe preeclampsia group according to the severity of HDCP, aiming to explore the changes in the miR-19a, miR-126, and miR-210 expression levels. We found that the miR-19a and miR-210 expression levels showed an upward trend along with the severity of the disease, indicating that the miR-19a and miR-210 expression levels were significantly increased in all stages of HDCP, and it was vice versa with miR-126. Previous studies found that [20] in the early stage of HDCP, the miR-19a expression can rise to more than 2 times its normal level, and the increase can be as high as 4 fold in severe preeclampsia. Some studies found that [21] that the down-regulation of specific microRNA-126 expressions will reduce angiogenesis and hypoxia in the right ventricle. Furthermore, a Pearson correlation analysis indicated that the serum miR-19a and miR-210 expression levels in HDCP patients were positively correlated with disease severity, and the miR-126 expression level was negatively correlated. Importantly, our ROC curve analysis showed that the area under the curve of the three combined was 0.896, and the sensitivity and specificity were 90.5% and 93.9%. Moreover, no statistically significant differences were observed in the areas under the curve between the three indicators. Taken together, these indicators have a higher specificity and sensitivity in HDCP. This highlights that there is a need to carry out early intervention measures based on the clinical diagnosis for these patients to reduce the incidence of HDCP. The potential mechanism is that the low expression of plasma miR-126 may be involved in the abnormal recasting of the uterine spiral artery, leading to an insufficient blood supply at the maternal-fetal interface, a reduction of effective placental perfusion, local tissue ischemia, hypoxia, and abnormal placental structure, which ultimately leads to vascular inflammation reactions and endothelial cell dysfunction. Additionally, miR-210 is a hypoxic activating factor. When the body is ischemic and hypoxic, the expression of miR-210 in tissues is up-regulated and promotes the formation of new blood vessels. A high expression of miR-210 in pregnant women can cause abnormal trophoblast cells, leading to an insufficient blood supply to the placenta [22]. Our study has several limitations due to the fact that it was hospital-based, with a small cohort. This needs to be addressed by expanding the cohort and conducting a multicenter, prospective study with a long-term follow-up.

In summary, miR-19a, miR-126, and miR-210 are strongly connected to disease severity in HDCP patients, and they can be used as sensitive indicators for predicting HDCP.

Disclosure of conflict of interest

None.

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