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Original Article

Homocysteine, endothelin-1 and nitric oxide in patients with hypertensive disorders complicating pregnancy

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Abstract: Objective: To investigate the change of level of serum homocysteine (Hcy), endothelin-1 (ET-1) and nitric oxide (NO) and clinical significance in patients with HDCP. Methods: Two hundred and thirty nine patients with HDCP (137 patients with mild preeclampsia, 102 patients with severe preeclampsia) who were hospitalized between June 2012 and June 2015 and 200 normal pregnancy women in outpatient department were enrolled in our study were divided into HDCP group and control group. Serum Hcy concentration was measured by enzymatic cycling assay. ET-1 concentration was measured by enzyme linked immunosorbent assay. And no concentration was measured by nitrate reductase assay. Results: Serum Hcy and ET-1 in HDCP group were significantly higher as compared to control group (P<0.05). Level of serum NO in HDCP group was significantly lower than in the control group (P<0.05). Level of serum Hcy and ET-1 in mild and severe preeclampsia group were significantly higher as compared to control group, respectively (P<0.05). Level of serum NO in mild and severe preeclampsia group were significantly lower than in the control group' respectively (P<0.05). Level of serum Hcy and ET-1 in severe preeclampsia group were significantly higher as compared to mild preclampsia group (P<0.05). Level of serum NO in severe preeclampsia group were significantly lower than in mild preeclampsia group (P<0.05). Spearman rank correlation analysis showed that level of serum Hcy and ET-1 was positively correlated with severity of diseases (r=0.689, 0.718, P<0.05). Level of serum NO was negatively correlated with severity of diseases (r=-0.702, P<0.05). Conclusion: Serum Hcy, ET-1 and NO were associated with pathogenesis of HDCP. Comprehensively measurement of them could effectively evaluate the incidence and progress of HDCP.

Keywords: Gestational hypertension, homocysteine, endothelin-1, nitric oxide

Introduction

Hypertensive disorders complicating pregnancy (HDCP), a pregnancy-specific disease with incidence rate of 9.4%~10.4% in China, is characterized as hypertension after 20 gestational weeks, elevated proteinuria and is a major cause of maternal and perinatal motality [1, 2]. To date, vascular endothelial cell dysfunction is suggested to be the most important key link affecting HDCP. Thus preventing endothelial cells from injury could effectively prevent and manage HDCP [3]. Elevated level of homocysteine (Hcy) which may injure endothelial cells is associated with the etiology of HDCP. Endothelin-1 (ET-1) and nitric oxide (NO) are contractive and relaxed vessel factors compounded and secreted by vascular endothelial cells and involve mediation of general and local vessel tension to play the role in vessel contraction and relaxation [4, 5]. There are few studies investigate the effect of Hcy, ET-1 and NO on the pathogenesis of HDCP. Therefore, we aim to investigate the mechanism of HDCP pathogenesis through detecting serum level of Hcy, ET-1 and NO in patients with HDCP.

Materials and methods

Clinical data

Two hundred and thirty nine patients with HDCP (137 patients with mild preeclampsia, 102 patients with severe preeclampsia) who were hospitalized between June 2012 and June 2015 and 200 normal pregnancy women in outpatient department were enrolled in our study were divided into HDCP group and control group. The study protocol was approved by ethical committee of our hospital. Inclusion criteria

Table 1. Demographic data in HDCP group and control group ($\bar{x}$±s)

Parameters	HDCP group (n=239)	Control group (n=200)	t	P
Age (years)	26.5±2.2	27.9±1.7	1.083	0.367
Gestational age (weeks)	35.5±1.6	36.1±2.0	0.731	0.692
Gravidity	1.3±0.5	1.1±0.7	0.922	0.518
Parity	0.9±0.3	1.2±0.2	1.012	0.391
BMI (kg/m^2)	22.1±1.5	21.0±1.7	0.894	0.615

Table 2. Serum Hcy, ET-1 and NO in HDCP group and control group ($\bar{x}$±s)

Parameters	HDCP group (n=239)	Control group (n=200)	t	P
Hcy (μmol/L)	14.52±1.33	8.21±1.54	4.237	0.015
ET-1 (ng/L)	72.38±5.15	46.75±4.97	5.091	0.026
NO (μmol/L)	31.30±2.98	45.04±3.34	5.382	0.029

included: (1) HDCP was defined as hypertension after midpregnancy (antenatal diastolic blood pressure ≥90 mmHg after 20 weeks' gestation in 2 or more readings at least 4 h apart) without proteinuria; preeclampsia was defined as hypertension after midpregnancy with proteinuria (≥1 protein reading on urine dipstick analysis or quantitative urine protein of ≥300 mg in a 24-h period). (2) Singleton pregnancy. All participants completed a medical history form and provided informed consent. Exclusion criteria included: Patients having cardiac dysfunction, liver and renal dysfunction, past hypertension history or coronary arteriosclerosis, acute or chronic nephritis or malignant tumor were excluded from study.

Methods

6 mL venous blood samples from elbow vein of participants with fasting in the morning were collected and 2 mL were put in test tube with BD vacuum separate gel. After 30 minutes of collections sample was centrifuged at 3000 rpm for 10 min to separate serum for Hcy measurement. Other 2 mL blood samples were put in anticoagulative tube with 20 μl EDTA-Na of 100 g/L and 40 μl apportioning. After sufficient mixing, the sample was centrifuged at 3000 rpm for 10 min to separate serum for ET-1 measurement. Other 2 mL blood samples were put in anticoagulative tube and centrifuged at 3000 rpm for 10 min to separate serum for NO measurement. All samples were stored at -70°C. Serum Hcy concentration was measured by enzymatic cycling assay. ET-1 concentration was measured by enzyme linked immunosorbent assay. And NO concentration was measured by nitrate reductase assay.

Statistical analysis

We performed all analyses by use of SPSS Software version 19.0. Numerical variables were reported in terms of mean and standard deviation ($\bar{x}$±s) and were analyzed between the 2 groups by using the Student t-test. Chi-square test was used to analyze comparison of independent sample of multiple groups. Pairwise comparison was analyzed by Dunnett-t test. And correlation of rank data was analyzed by Spearman rank correlation analysis. Variables showing *P*-value less than 0.05 were considered to be statistically significant difference.

Results

Demographic data in HDCP group and control group

Age, gravidity, parity and BMI of patients in the two groups were comparable (*P*>0.05) (**Table 1**).

Serum Hcy, ET-1 and NO in HDCP group and control group

Serum Hcy and ET-1 in HDCP group were significantly higher as compared to control group (*P*<0.05). Level of serum NO in HDCP group was significantly lower than in the control group *(P*<0.05) (**Table 2**).

Serum Hcy, ET-1 and NO in different type of HDCP group and control group

There were statistically significant differences among the three groups in terms of serum Hcy, ET-1 and NO (*P*<0.05), respectively. Level of serum Hcy and ET-1 in mild and severe preeclampsia group were significantly higher as compared to control group, respectively (*P*< 0.05). Level of serum NO in mild and severe preeclampsia group were significantly lower than in the control group, respectively (*P*<0.05). Level of serum Hcy and ET-1 in severe preeclampsia group were significantly higher as compared to mild preclampsia group (*P*<0.05).

Table 3. Serum Hcy, ET-1 and NO in different type of HDCP group and control group ($\bar{x}$±s)

Parameters	Mild preeclampsia (n=127)	Severe preeclampsia (n=102)	Control group (n=200)	t	P
Hcy (μmol/L)	12.72±1.29※	16.05±1.46※,#	8.21±1.54	11.895	0.018
ET-1 (ng/L)	65.83±5.56※	79.34±5.08※,#	46.75±4.97	10.532	0.027
NO (μmol/L)	36.23±2.97※	27.59±3.81※,#	45.04±3.34	9.778	0.031

Note: ※Compared with mild preeclampsia and control group, P<0.05. ※,#Compared with mild preeclampsia group, P<0.05.

Level of serum NO in severe preeclampsia group were significantly lower than in mild preeclampsia group (P<0.05) (**Table 3**).

Correlation between severity of HDCP and serum Hcy, ET-1 and NO

Spearman rank correlation analysis showed that level of serum Hcy and ET-1 was positively correlated with severity of diseases (r=0.689, 0.718, *P*<0.05). The more serious is severity of diseases, the higher is the level of serum Hcy and ET-1. Level of serum NO was negatively correlated with severity of diseases (r=-0.702, *P*<0.05). With the increase severity of diseases, level of serum NO was decreased.

Discussion

As a common pregnancy-specific disease of Obstetrics, HDCP is an extremely serious hiHDCP risk for cause of poor outcome of pregnancy women and fetus. Studies have indicated that systematic vasospasm occurs in pregnancy women with preeclampsia resulting in hemoconcentration and increased resistance of peripheral vessels. When severe preeclampsia is developed, pregnancy women and fetus may be dead [7-9]. It is very important to investigate the pathogenesis of HDCP and take action to prevent preeclampsia with effect measures according to pathogenesis so that mortality of pregnancy women and fetus can be reduced and life quality is increased. To date studies have indicated that vascular endothelial injury caused by vascular inflammatory including participants of cytokines, inflammatory factors and coagulation system has become the centerpiece in the contemporary understanding of the pathogenesis of preeclampsia [10, 11].

Hcy is a sulphur amino acid of intermediate product of methionine metabolism. Hcy is easily oxidized to homocysteic acid compound and free radical of superoxide anion and hydrogen peroxide are produced leading to injury of vascular endothelial cells. Thus Hcy is an important indicator reflecting injury of vascular endothelial cells [12, 13]. The present study shows that level of serum Hcy in HDCP group was higher as compared to normal pregnancy group. And level of serum Hcy in severe preeclampsia group was higher as compared to mild preeclampsia group. These results indicated that there is close relationship between Hcy and HDCP, and with the severity of diseases increasing, level of serum Hcy is increased, which is consistent with the results of other studies [14]. The reason might be that metabolism need is increased in pregnancy period. But because of insufficient intake or malabsorption of B group vitamins and folic, level of serum Hcy is increased resulting in injury of vascular endothelial cells.

ET-1 is a powerful vasoconstrictor peptide which is produced by endothelial cell and could promote proliferation of smooth muscle cell and vascular contraction. Studies have shown that hypertension and inflammatory factors could damage intact structure of endothelial and promote the synthesis of ET-1 which is released to blood resulting in systematic vasospasm and increased resistance of renal vessels. At the same time, aldosterone and angiotesin are secreted to make blood pressure elevated, subsequently accelerate injury of endothelial cells [15-17]. NO has the properties of relaxing vascular smooth muscle, inhibiting adhesion and aggregation of platelet and anticoagulation. Its synergistic effect with ET-1 could mediate function of endothelial. Studies have found that increased level of NO could effectively maintain the status of hiHDCP blood flow and hypotension in pregnancy, consequently, vascular tension of placenta is maintained so that fetus could grow normally [18]. The results of present study show that level of serum Hcy in HDCP group was higher as compared to normal pregnancy group. And level of serum Hcy in severe preeclampsia group was higher as compared to mild preeclampsia group. But level of serum NO in HDCP group is

lower than in the control group. And level of serum NO in severe preeclampsia group was lower as compared to mild preeclampsia group. The results above indicated that ET-1 and NO play an important role in pathogenesis of HDCP.

The results of present study show that level of serum Hcy in patient with HDCP was positively correlated with severity of disease. And the more serious was the disease, serum Hcy was higher. These results further proved that Hcy involved in the pathogenesis of HDCP. It was reported that ET-1 could aggravate hypoxia of endothelial and increase injure of endothelial function. Meanwhile, the pressure rising of angiotesin induced by ET-1 and the pressure rising of itself could increase significantly blood pressure, subsequently preeclampsia is aggravated [19, 20]. The present study has similar results that level of serum ET-1 was positively correlated with severity of HDCP, which indicated that ET-1 involves in the pathogenesis of HDCP. The present study also found that level of serum NO was negatively correlated with severity of HDCP. With the severity of disease increasing, level of serum NO was decreased, which suggested that NO play an important role in pathogenesis of HDCP.

All in all, injury of vascular endothelial cells is the important factor for HDCP, whereas serum Hcy, ET-1 and NO which play an important role in injury of vascular endothelial cells involves in pathogenesis of HDCP. Thus they can be an important reference indicator for prevention of HDCP in clinical practice. Though our study has provided guidance for further research and clinical practice, there are still some limitations. For example, we didn't follow up all patients and could not get the data of serum Hcy, ET-1 and NO after recovery and pregnancy outcomes. Therefore, follow-up data should be collected in future studies in order to get more strong evidence for clinical practice.

Disclosure of conflict of interest

None.

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