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Research Paper

The investigation for the relationship among serum leptin, erythrocyte membrane Ca^{2+}-ATPase activity and hypertensive disorder complicating pregnancy

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Abstract

Objective: To study the significance of Leptin and the activity of erythrocyte membrane Ca^{2+}-ATPase(EMCA) in the development of hypertensive disorder complicating pregnancy. **Methods**: Radioimmunoassay was used to test the level of serum Leptin, and the activity of EMCA was determined chemically in 38 pregnant women with hypertensive disorder complicating pregnancy and 36 normotensive pregnant women. **Results**: The level of serum Leptin in hypertensive disorder complicating pregnancy(gestational hypertension: 13.76 ± 3.46 ng/ml; preeclampsia: 15.76 ± 5.47 ng/ml; eclampsia: 18.32 ± 6.38 ng/ml)was significantly higher than that in normotensive pregnant women (11.33 ± 2.93 ng/ml), respectively. The average EMCA activity of patients with hypertensive disorder complicating pregnancy (gestational hypertension: 1.65 ± 0.24 μmol·pi/mg·h ; preeclampsia: 1.37 ± 0.19 μmol·pi/mg·h; eclampsia: 1.12 ± 0.14 μ mol·pi/mg·h) was significantly lower than that of normotensive pregnant women(1.83 ± 0.38 μ mol·pi/mg·h), respectively. There was a negative correlation between the level of serum Leptin and the activity of RMCA in hypertensive disorder complicating pregnancy ($r = -0.63$). **Conclusion**: Inhibition of EMCA activity of erythrocyte in hypertensive disorder complicating pregnancy may increase cytoplasmic free calcium, which contributes to the development of hypertensive disorder complicating pregnancy. The negative correlation between the level of serum Leptin and the activity of EMCA, also suggested that serum Leptin and the activity of EMCA may play a role in the development of hypertensive disorder complicating pregnancy.

Keywords: Leptin; erythrocyte membrane Ca^{2+}-ATPase activity; hypertensive disorder complicating pregnancy

INTRODUCTION

Hypertensive disorder complicating pregnancy is a well documented complication occurring during pregnancy, which severely threatens the health of mother and fetus. The etiologies are not clear yet, however several recent researches found that the level of serum Leptin in gravida with the hypertensive disorder, as elevated. This suggests potential relationship between Leptin and the condition. The concentration of intracellular Ca^{2+} also elevates in patients, which is caused mainly by decreasing activity of erythrocyte membrane Ca^{2+}-ATPase activity (EMCA), partially receiving the modulation of insulin. The secretion of Leptin is also linked with insulin level. This study aimed to discuss the significance of Leptin and the activity of EMCA in the development of hypertensive disorder complicating pregnancy.

MATERIALS AND METHODS

Subjects

38 cases of hypertensive disorder complicating pregnancy were admitted to the obstetric and gynecology department of the First Affiliated Hospital, Xi'an Jiaotong University (observation group). Of which, 16 cases were classified as having gestational hypertension, 16 cases classified with preeclampsia, and 6 cases as having eclampsia. 36 cases normal

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pregnancy women were admitted during the same period and designated as control group. Average pregnancy duration of patients in our observation group was 36.4 ± 1.8 weeks, and 37.4 ± 2.8 weeks in control group. The average pre-gestational weight index were 19.6 ± 2.4 in the case group and 18.9 ± 1.6 in normal group. None of the patients was in labor when admitted, none with a known smoking history during pregnancy, with no history of anti-hypertension drug or calcium agents involved around the period of conception or pregnancy. The diagnosis and classification standards were according to *Obstetric and Gynecology (6th edition)*.

Methods

A blood sample of 2 ml was collected from the elbow vein in the morning, on an empty stomach after admission, subsequently the serum was separated and stored at −70℃ until analysis. Radioimmunoassay was used to test the level of serum Leptin. The test kit was provided by Tianjin Xiehe medical and pharmacology technological limited company. All steps followed the instructions strictly, with a 5 ml blood sample collected at the same time to separate red blood cell after using heparin. The red blood cell membrane were then prepared for evaluating the activity of calcium adenosine triphosphatase as described previously[1,2].

Statistical analysis

All data were present as $\bar{x} \pm s$ and analyzed with SPSS10.0 software pack. The significant difference was set at $P < 0.05$ after calculating the average, using *t*-test and direct correlated regression analysis.

RESULTS

Serum levels of Leptin in each group

Hypertensive disorder complicating pregnancy group was significantly higher than control group in serum level of leptin ($P < 0.01$)(***Tab 1***).

The activity of EMCA in each group

Hypertensive disorder complicating pregnancy group was significantly lower than control group in the activity of EMCA($P < 0.01$) (***Tab 2***).

Tab 1 **Serum level of Leptin in hypertensive complicating gravida and normal gravida** ($\bar{x} \pm s$)

Group	Cases	Leptin(ng/ml)
Control	36	11.33 ± 2.93
Gestational Hypertension	16	13.76 ± 3.46*
Preeclampsia	16	15.76 ± 5.47**
Eclampsia	6	18.32 ± 6.38**

Compared with the control group: *$P < 0.05$; **$P < 0.01$.

Tab 2 **The activity of EMCA in hypertensive disorder complicating pregnancy gravida and normal gravida** ($\bar{x} \pm s$)

Group	Cases	EMCA(μ mol·pi/mg h)
Control	36	1.83 ± 0.38
Gestational Hypertension	16	1.65 ± 0.24*
Preeclampsia	16	1.37 ± 0.19**
Eclampsia	6	1.12 ± 0.14**

Compared with the control group: *$P < 0.05$; **$P < 0.01$.

The correlation between the level of serum Leptin and the activity of EMCA in hypertensive disorder complicating pregnancy

There was a negative correlation between the level of serum Leptin and the activity of EMCA in hypertensive disorder complicating pregnancy ($r = -0.63$).

DISCUSSION

Leptin and hypertensive disorder complicating pregnancy

It is well known that obesity is a common disorder, associated with various metabolic and cardiovascular diseases, such as diabetes, dyslipidemia, hypertension, stroke, arteriosclerosis, coronary heart diseases, and congestive heart failure [3,4]. In 1994, Zhang et al successfully cloned the fat gene and its coding product Leptin. Recent findings demonstrated that serum Leptin level is elevated in primary hypertension and that Leptin could trigger the activity of sympathetic nerve. This activity was found higher in gravida with hypertensive disorder. So is believed to participate in the procedure of onset and development of hypertensive disorder complicating pregnancy. Mise et al found that Leptin mRNA expression increased in placenta tissues of patients with hypertensive disorder, and it showed a positive correlation with the level in serum. Placenta hypoxia was the main reason for the increase either of Leptin synthesis in placenta, or releasing in serum. This Leptin increment could inhibit the secretion of one protein NPY, which probably has the potential function of vessel relaxation. As a result, Leptin increment may have a contribution to induced placenta vessel contraction. Additionally, researches confirmed that NPYmRNA expression declined, resulting in placenta hypoxia and partially owing to the occurrence of hypertensive disorder complicating pregnancy. Admittedly, there still were controversial results. Leptin mRNA expression in placenta didn't show difference between patients with hypertensive disorder and normal pregnancy women[5]. But serum

free Leptin in gravida with hypertensive disorder declined. Ning *et al* [6] found that serum Leptin level in preeclampsia patients significantly elevated and showing a positive correlation with weight index. The incidence of hypertensive disorder increased in women with obesity and higher serum Leptin level, especially in first trimester. Serum Leptin level stepped up further at 20 gestational weeks in pregnant women with hypertensive disorder complicating pregnancy. Hendler *et al* [7] studied the level of Leptin in pre-eclampsia patients with differing BMI. They stated that the Leptin level increased in gravida with BMI >= 25 kg/m^2. Chan TF *et al* [8] established that the Leptin level of amion fluid in preeclampsia patients was higher than in normal pregnancy women and its concentration decreased as gestational age advanced. All of these indicate a close relationship between Leptin and hypertensive disorder during pregnancy. Moreover, interestingly, the elevation of Leptin serum level was in advance of the presentation of abnormal clinical manifestation. Recent studies[9] reported that Leptin might become a predictable index to hypertensive disorder complicating pregnancy. The risk of hypertensive disorder during pregnancy was found increased if the Leptin ratio of amino to mother blood was elevated in the primary trimester. This study found that serum Leptin level in patients with hypertensive disorder complicating pregnancy was much higher than normal gravida and became higher as the condition became worsen. It suggested that serum Leptin level could be a biomarker to reflect the severity of hypertensive disorder complicating pregnancy.

The relationship between calcium adenosine triphosphatase of cell membrane and hypertensive disorder complicating pregnancy

Previous studies found that the concentration of intracellular free Ca^{2+} was elevated and serum Ca^{2+} lowered in gravida with hypertensive disorder complicating pregnancy. The increment of intracellular free Ca^{2+} led to smooth muscle cell contraction, arteriole spasm and then hypertension. Calcium adenosine triphosphatase of cell membranes was an important factor to modulate the distribution of Ca^{2+}, which mainly contributes to intracellular free Ca^{2+} elevation. When the activity of calcium adenosine triphosphatase of cell membrane decreased, outflowing of Ca^{2+} was blocked. The intracellular Ca^{2+} increased as a result. Therefore, it was supposed that overload of intracellular Ca^{2+} was due to the low activity of calcium adenosine triphosphatase of the cell membrane. Red blood cell membrane has both the active and passive transportation systems of calcium. So red blood cell membrane is highly relevant for experiment of calcium-channel study. Oviedo NJ *et al* [10] hold the statement that plasma membrane Ca^{2+}-ATPase activity diminishes by about 50% in red blood cells during preeclampsia. It was found that the phosphorylated intermediate associated with EMCA is similar for red cell ghosts from normotensive and preeclamptic women, suggesting a similar number of ATPase molecules in these membranes. The molecular weight of the Ca^{2+}-ATPase is around 140 kDa for both normotensive and preeclamptic membranes, and its cross-reactions with specific antibodies is also similar, suggesting that the protein structure remains intact in preeclampsia. Their results showed that the reduced Ca^{2+}-ATPase activity of the red cell membranes from preeclamptic women is not associated with a defective enzyme, but rather with a high level of lipid peroxidation. Nardulli et al studied the activity of calcium adenosine triphosphatase of red blood cell membrane in preeclampsia women. They discovered that it reduced 50% comparing with normal gravida. Accordingly, they suspected that low perfusion of uterus-placenta, tissue hypoxia and lipid peroxidation were responsible. Carrera *et al*[11] evaluated the activity of calcium adenosine triphosphatase of uterus muscle cell and trophoblastic cells. They concluded the activity of calcium adenosine triphosphatase of cell membrane decreased in women with preeclampsia, due to the increment of acid products of lipid peroxidation. Others found [12] that EMCA recovered after labor in women with preeclampsia. EMCA returned to normal at the termination of pregnancy and improvement of the condition. This confirmed its close relation to hypertensive disorder complicating pregnancy. This study discovered that the activity of calcium adenosine triphosphatase of red blood cell membrane decreased, and showed a relation with the severity of the condition. It illustrated decreasing of calcium adenosine triphosphatase of red blood cell membrane caused intracellular Ca^{2+} increasing, vessel smooth muscle contraction and arteriole spasm leading to the manifestation of hypertension.

The relationship between serum Leptin level and the activity of calcium adenosine triphosphatase in the red blood cell membrane in patients with hypertensive disorder complicating pregnancy

This study discovered that serum Leptin level and

the activity of calcium adenosine triphosphatase of the red blood cell membrane in patients with hypertensive disorder complicating pregnancy had a significant correlation ($r = -0.63$), because both of them were related with the modulation of insulin. Many researches in recent years found[13] that the expression and secretion of Leptin mRNA were modulated by insulin. These were positive related. Insulin plays key role in monitoring of serum Leptin level. It stimulates the expression of Leptin in fat cells *in vitro*, dose-dependently. There were significant hyperinsulinism and insulin resistance in patients with hypertensive disorder complicating pregnancy. Seely *et al*[14] indicates relations between serum Leptin and insulin level, serum Leptin level and insulin sensitivity in patients with hypertensive disorder complicating pregnancy. Both insulin level and sensitivity were the important determining factors for hypertensive disorder complicating pregnancy. The activity of calcium adenosine triphosphatase in the red blood cell membrane was also controlled by insulin. Physiologically, insulin could promote the activity of calcium adenosine triphosphatase of red blood cell membranes, of which has weakened function in hypertensive disorder complicating pregnancy due to insulin resistance. The results confirmed two hypotheses about etiology of hypertensive disorder in pregnancy and their correlations: placenta hypoxia and imbalance of extra and intracellular calcium.

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