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Effect of pancreatic kallikrein combined with magnesium sulfate therapy on disease severity of patients with severe preeclampsia

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ABSTRACT

Objective: To study the clinical effect of pancreatic kallikrein combined with magnesium sulfate therapy on severe preeclampsia. **Methods:** A total of 47 patients with severe preeclampsia treated in our hospital from May 2013 to October 2015 were retrospectively analyzed, patients who received pancreatic kallikrein combined with magnesium sulfate therapy were selected as observation group, patients who received magnesium sulfate therapy were selected as control group, and then blood pressure level, renal function, uterine artery and umbilical artery blood flow state, and hypoxia indexes in serum and placenta were compared between two groups. **Results:** After treatment, systolic pressure and diastolic pressure levels as well as serum CysC level and 24 h urine protein level of observation group were significantly lower than those of control group, the uterine artery and umbilical artery S/D and PI were significantly lower than those of control group, PIGF and NO levels in serum as well as Xiap and Survivin levels in placenta were significantly higher than those of control group, and sVEGFR1 level in serum as well as Caspase-3 and Caspase-7 levels in placenta was significantly lower than those of control group. **Conclusions:** Pancreatic kallikrein combined with magnesium sulfate therapy can alleviate the disease severity, lower blood pressure and vascular resistance, and improve renal function and the apoptosis caused by placental hypoxia in patients with severe preeclampsia.

1. Introduction

Preeclampsia is a common complication during pregnancy and also an important cause that can affect the health of the pregnant and the fetal. The most typical pathological physiological changes of the disease are the systemic arteriolar spasm as well as placenta tissue and renal hypoperfusion, characterized by hypertension and proteinuria[1,2]. Magnesium sulfate is a common drug for the clinical treatment of preeclampsia, has the function of relaxing smooth muscle, and thus can reduce the vascular resistance in pregnant women with preeclampsia to a certain extent, thereby lowering blood pressure levels. However, the overall efficacy of magnesium sulfate alone is not satisfactory in the treatment of preeclampsia, and most patients still need to terminate pregnancy to ensure the safety of the maternal and the fetal. Kallikrein is involved in the composition of kallikrein-plasmakinin system in the body, and can split the kininogen into active plasmakinin, thus exerting the effect of relaxing blood vessels, reducing blood flow resistance and improving microcirculation perfusion[3,4]. At present, the pancreatic kallikrein is used to treat microvascular complications caused by hypertension, diabetes and other diseases[5,6], but the value of the drug for treatment of preeclampsia has not been clearly reported yet. In the following study, the clinical value pancreatic kallikrein combined with magnesium sulfate therapy for severe preeclampsia was analyzed.

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2. Materials and methods

2.1. Research subjects

Patients with severe preeclampsia treated in our hospital from May 2013 to October 2015 were selected as the research subjects, all the patients conformed to the diagnostic criteria for severe preeclampsia, their medical records were complete, serum samples had been collected before delivery, and placental tissue samples had been collected within 30 min after delivery. Patients whose medical records were not complete or serum specimens and placental tissue samples were not collected were ruled out. A total of 47 patients were included, their medical records were reviewed and according to different drug treatment, they were divided into observation group and control group. Observation group 22 cases received pancreatic kallikrein combined with magnesium sulfate treatment, they were (29.4±4.2) years old and the gestational age at delivery was (35.9±5.2) weeks; control group 25 cases received magnesium sulfate therapy, they were (29.8±4.7) years old and the gestational age at delivery was (36.3±5.7) weeks. Two groups showed no significant difference in general data.

2.2. Treatment methods

Observation group: pancreatic kallikrein 40 U dissolved in 1.5 mL water for injection, intramuscular injection and 2 times/day, 30 mL of 25% magnesium sulfate added in 20 mL of normal saline injection, by micro-pump injection and 2 times/day. Control group: 30 mL of 25% magnesium sulfate added in 20 mL of normal saline injection, by micro-pump injection and 2 times/day. Blood pressure and fetal heart conditions were monitored during treatment and the treatment lasted for 3 consecutive days.

2.3. Clinical index evaluation

2.3.1. Illness-related indexes

Three days after treatment, blood pressure levels of two groups were detected and recorded; 24 h urine specimens were kept to determine protein levels; fasting peripheral blood samples were collected and centrifuged to separate serum, and then enzyme-linked immunosorbent assay kits were used to determine cystatin C (CysC), placental growth factor (PIGF), soluble vascular endothelial growth factor receptor 1 (sVEGFR1) and nitric oxide (NO) levels.

2.3.2. Apoptosis molecule expression levels in placenta tissue

Within 30 min after the delivery of placenta, 1 cm^3 of tissue block was collected from the central zone of the maternal placenta, obvious hemorrhagic spots and calcifications should be avoided, and the placenta tissue was washed with normal saline for 5 times and then cryopreserved in liquid nitrogen. For detection, the placenta tissue was taken out, about 30 mg of tissue was weighed, added to PBS and then fully homogenized, the homogenate was centrifuged in 4 ℃ centrifugal machine for 20 min to separate supernatant, and enzyme-linked immunosorbent assay kits were used to determine Xiap, Survivin, Caspase-3 and Caspase-7 levels in supernatant.

2.4. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis between two groups was performed by t test and $P<0.05$ indicated statistical significant differences.

3. Results

3.1. Illness-related indexes of preeclampsia

Systolic pressure and diastolic pressure levels of observation group after treatment were significantly lower than those of control group ($P<0.05$); analysis of renal function indexes of two groups was as follows: serum CysC and 24 h urine protein levels of observation group after treatment were significantly lower than those of control group ($P<0.05$).

Table 1
Illness-related indexes of two groups.

Groups	Case No.	Blood pressure levels (mmHg)		Renal function indexes	
		Systolic pressure	Diastolic pressure	Serum CysC (mg/L)	24 h urine protein (g)
Observation group	22	129.2±18.8	81.3±10.2	0.82±0.11	1.01±0.14
Control group	25	134.3±17.1	89.4±9.4	1.25±0.18	1.63±0.22
t		6.822	7.103	7.684	6.937
P		<0.05	<0.05	<0.05	<0.05

3.2. Uterine artery and umbilical artery blood flow state

The uterine artery and umbilical artery S/D and PI of observation group were significantly lower than those of control group ($P<0.05$) (Table 2).

Table 2
Ultrasound parameters of uterine artery and umbilical artery of two groups.

Groups	Case No.	Uterine artery		Umbilical artery	
		S/D	PI	S/D	PI
Observation group	22	1.93±0.22	0.82±0.10	2.32±0.41	0.91±0.12
Control group	25	2.41±0.33	1.03±0.14	2.79±0.39	1.14±0.16
t		6.822	5.924	6.357	6.751
P		<0.05	<0.05	<0.05	<0.05

3.3. Serum placental hypoxia-related indexes

Serum PIGF and NO levels of observation group after treatment were significantly higher than those of control group, sVEGFR1 level was significantly lower than that of control group ($P<0.05$) (Table 3).

Table 3
Serum PIGF, sVEGFR1 and NO levels of two groups.

Groups	Case No.	PIGF (pg/mL)	sVEGFR1 (pg/mL)	NO (μmol/L)
Observation group	22	574.21±75.23	2351.32±366.75	84.65±9.32
Control group	25	342.35±56.37	3418.97±525.61	62.39±8.24
t		8.918	7.548	6.951
P		<0.05	<0.05	<0.05

3.4. Hypoxia-induced apoptosis in placenta

Xiap and Survivin levels in placenta of observation group after treatment were significantly higher than those of control group, Caspase-3 and Caspase-7 levels were significantly lower than those of control group ($P<0.05$) (Table 4).

4. Discussion

Systemic arteriolar spasm is the basic pathological physiological characteristic of pregnant women with preeclampsia, and can lead to elevation of blood pressure as well as placental and renal hypoperfusion. Magnesium sulfate has the function of relaxing smooth muscle, is used in the treatment of preeclampsia, and can relax blood vessels, lower blood pressure and improve renal and placental blood perfusion to a certain extent. However, the curative effect of magnesium sulfate alone is not ideal for treatment of severe preeclampsia, and the placental ischemia hypoxia is relatively severe and will synthesize a variety of molecules with damage effect, resulting in maternal endothelial dysfunction. The pancreatic kallikrein used in the study is involved in the composition of kallikrein-plasmakinin system in the body, and can split the kininogen into vasoactive plasmakinin, which plays the role of relaxing blood vessels, lowering blood pressure and improving viscera blood perfusion[7,8]. In the study, blood pressure levels of patients with preeclampsia were analyzed after treatment at first, and the systolic pressure and diastolic blood pressure levels of observation group were significantly lower than those of control group. This means that pancreatic kallikrein combined with magnesium sulfate treatment can reduce the blood pressure levels of patients with severe preeclampsia.

Renal injury is an important clinical feature in patients with preeclampsia, and arteriolar spasm will affect renal blood perfusion and cause glomerular damage. In addition, the placental hypoperfusion caused by uterine arterial and umbilical arterial spasm can cause increased synthesis of a variety of damage molecules, which can cause glomerular injury after released into the blood circulation[9]. Glomerular damage will cause that the protein in blood circulation is filtered out by the glomeruli and discharged with the urine, and it will also influence the scavenging effect of the glomeruli on metabolites *in vivo*. CysC is small-molecule substance discharged by the glomeruli, and glomerular damage of patients with preeclampsia will affect the excretion of CysC and cause CysC accumulation and elevated serum CysC content in the body. In the study, glomerular damage level as well as uterine artery and umbilical artery blood flow state of two groups was analyzed, and the results showed that serum CysC and 24 h urine protein levels as well as the uterine artery and umbilical artery S/D and PI of observation group were significantly lower than those of control group. This means that pancreatic kallikrein combined with magnesium sulfate therapy can improve the glomerular function and increase the uterine artery and umbilical artery flow perfusion.

Increased uterine artery and umbilical artery resistance and decreased blood flow in patients with preeclampsia can cause placental hypoperfusion, and placental tissue is in a state of ischemia hypoxia and will lead to local abnormal synthesis of a variety of molecules[10,11]. PIGF is a new member in VEGF family that can promote the placental angiogenesis and increase vascular permeability, and meanwhile, can be secreted into maternal blood circulation and protect the endothelium[12,13]. SVEGFR1 can be secreted into the blood circulation in soluble form to antagonize the angiogenesis-promoting and endothelial protective function of PIGF, VEGF and other molecules, influence the formation of new blood vessels within the placenta and cause maternal endothelial function damage[14,15]. NO is an important vasodilatory molecule in the body and placental hypoxia can affect the synthesis and release of NO[16]. In the study, analysis of serum levels of placental hypoxia-related molecules of two groups proved that serum PIGF and NO levels of observation group after treatment were significantly higher than those of control group, and sVEGFR1 level was significantly lower than that of control group. It means the pancreatic kallikrein combined with magnesium sulfate therapy can increase placental perfusion to adjust the generation and release of PIGF, sVEGFR1, NO and other molecules.

Finally, in order to further clarify the molecular changes of placenta in hypoxic conditions, the expression levels of apoptosis-related molecules in the placenta were detected after treatment. XIAP and Survivin are two important anti-apoptotic molecules in the placenta, both belong to the IAP family, and have antagonism effect on the release of cytochrome C in the mitochondria, thereby inhibiting the activation of downstream Caspase-3, Caspase-9 and other

Table 4
Hypoxia-induced apoptosis in placenta of two groups (ng/mL).

Groups	Case No.	Xiap	Survivin	Caspase-3	Caspase-9
Observation group	22	69.29±8.94	105.96±22.10	1.94±0.35	11.58±3.85
Control group	25	33.14±5.59	64.33±7.94	4.17±0.74	34.28±6.93
t		11.833	9.039	15.833	18.691
P		<0.05	<0.05	<0.05	<0.05

apoptosis-promoting molecules[17]. Ischemia hypoxia conditions can inhibit the expression of XIAP and Survivin, and weaken the anti-apoptosis ability of local tissue. In the study, the analysis proved that Xiap and Survivin levels in placenta of observation group after treatment were significantly higher than those of control group while Caspase-3 and Caspase-7 levels were significantly lower than those of control group. This means that pancreatic kallikrein combined with magnesium sulfate therapy can inhibit the apoptosis caused by placental hypoxia.

To sum up, pancreatic kallikrein combined with magnesium sulfate therapy can alleviate the disease severity, lower blood pressure and vascular resistance, and improve renal function and the apoptosis caused by placental hypoxia in patients with severe preeclampsia.

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