



Analysis on Clinical Outcomes of Low-Molecular Weight Heparin Combined with Magnesium Sulfate in Patients with Pre-Eclampsia

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Abstract

Background: We aimed to investigate the clinical effect of low molecular weight heparin (LMWH) combined with magnesium sulfate in patients with severe pre-eclampsia (PE).

Methods: A total of 70 patients with severe PE admitted in Suzhou Kowloon Hospital, Shanghai Jiao Tong University School of Medicine, China from Jun 2020 to May 2021 were enrolled and randomized into observation and control groups. The control group was treated with magnesium sulfate only, and the observation group was treated with small-dose LMWH combined with magnesium sulfate. The clinical effects and artery blood flow index were analyzed.

Results: The observation group had higher coagulation function than that in the control group. RI, S/D, and PI in the observation group after treatment were lower than those in the control group, with statistically significant differences ($P < 0.05$). In serum and placental tissues, both kininase expression and TGF-1 levels of the observation group were higher than those of the control group, with lower PAPP-A, VCAM-1 and E selectin levels than those in the control group.

Conclusion: Small-dose LMWH combined with magnesium sulfate can promote the expression of endogenous kallikrein, weaken the infiltration of cytotrophoblast, and improve the maternal blood clotting state in patients with severe PE. It also improves placental microblood flow and effectively prolong pregnancy, promotes fetal growth, and improves the clinical effects.

Keywords: Pre-eclampsia; Low molecular weight heparin; Magnesium sulfate; Coagulation function; Clinical effect

Introduction

Pre-eclampsia (PE) is one of the multiorgan damaging diseases characterized by hypertension

(systolic pressure ≥ 160 mmHg or diastolic pressure ≥ 110 mmHg) and proteinuria $\geq 0.3\text{g}/24\text{h}$



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after 20 weeks of pregnancy, which can endanger the mother's life in severe cases, and its pathogenesis has not been clarified (1,2). According to statistics, up to 10% of pregnancies are affected by hypertension diseases such as PE (3-6). Correlation studies showed that the main pathogenesis of PE is insufficient uterine spiral artery recast, placental dysfunction, overactivation of inflammation and damaged vascular endothelial cells. The central links of the basic pathophysiological changes include spasm of small blood vessels and damage of vascular endothelial cells (7-11). Injury to the blood pressure endothelium due to neutrophil activation and adhesion to vascular endothelial cells to stimulate an inflammatory response is the major pathophysiological change of PE (12, 13). Signaling pathways, such as Mitogen-activated protein kinase (MAPK) and downstream target protein kinase (ERK1/2, and JNK) participate in the regulation of neutrophils activation process (14).

Low molecular weight heparin (LMWH) has functions of anti-coagulation, anti-inflammatory, protecting vascular endothelial cells, reducing blood viscosity, regulating cell proliferation, and improving placental microcirculation (15). Application of LMWH in patients with early PE can reduce the recurrence rate of PE and improve the adverse outcome of mother and baby in pregnancy (16,17). LMWH plays a role in anti-coagulation and anti-thrombus, promoting the proliferation of trophoblast cells, and regulating immunity in early PE. And it has certain positive significance to improving the prognosis of mothers with PE and their babies with high safety in its application.

For patients with severe PE who must undergo active intervention and early termination of pregnancy, magnesium sulfate is the preferred drug to prevent severe PE, with the functions of spasmolysis and controlling eclamptic convulsions. However, the effective blood magnesium concentration for the treatment is very narrow, and it will lead to respiratory, liver and kidney function damage and even sudden death when it is too high. Therefore, magnesium sulfate cannot be used for a long time with large amount (18).

To sum up, the early treatment of severe PE was mainly spasmolysis and antihypertensive, which, however, cannot improve the hypercoagulation state of blood (19-21). Although the development of the disease can be controlled, simple magnesium cannot achieve the expected effects, which is easy to increase a series of complications. As the increasing application in clinical treatment, LMWH combined with magnesium sulfate can effectively relieve small blood vessel spasms and improve the prognosis of newborns. Therefore, in this study, patients with severe PE were treated with LMWH combined with magnesium sulfate.

Materials and Methods

Subjects

A total of 70 patients with severe PE admitted to Suzhou Kowloon Hospital, Shanghai Jiao Tong University School of Medicine, China from Jun 2020 to May 2021 were enrolled. All pregnant women had regular menstrual cycle and clear final menstruation, without primary hypertension, liver and kidney diseases, cardiovascular and cerebrovascular diseases, and history of smoking and drinking. None of them had received spasmolysis and antihypertensive treatment before admission.

This study has been approved by the Ethics Committee of Suzhou Kowloon Hospital. Informed consent has been signed and obtained from all patients.

Inclusion criteria

- 1) All patients met the diagnostic criteria for severe PE: blood pressure $\geq 160 / 110$ mmHg; urine protein ≥ 300 mg/24 h or random urinary protein (+); platelets $< 100 \times 10^9 / L$; Serum ALT or AST (+); and may be accompanied by upper abdominal discomfort, headache and other symptoms;
- 2) All patients have blurred vision, headache, coma, etc.;
- 3) The medical records were clear and complete;
- 4) Single fetus

5) All patients have knowingly signed informed consent forms.

Exclusion criteria

- 1) Patients who were allergic to or allergic to heparin;
- 2) Patients with multicoagulation dysfunction, multiple organ dysfunction and so on;
- 3) Patients with severe heart, liver failure and kidney failure;
- 4) Patients with fetal chromosomal abnormalities or fetal abnormalities.

Treatment method

Routine treatment was given to patients of two groups. The control group was treated with magnesium sulfate as follows: 20 mL of 25% magnesium sulfate was added to 100 mL of 5% glucose for intravenous infusion, controlling infusion rate at 30 drops/min, which was completed within 30 min, with the total daily dosage of magnesium sulfate not more than 30 g. After that, 60 mL of 25% magnesium sulfate was dissolved in 1000 mL of 25% glucose solution for intravenous infusion, with the infusion rate at 2 g/h, the daily dosage of 20 g, 1 time/d, and 5 days were taken as 1 course, which was continued to the end of patient delivery. After venous magnesium sulfate for 48 h, the maternal heart rate, systolic pressure, diastolic pressure and mean arterial pressure were recorded, and the patient was observed for vital signs, and terminated the pregnancy immediately if necessary.

The observation group was treated with a small dose of LMWH for 1 time/d, with subcutaneous injection. When taking medication, the patient's heart rate, breathing frequency, urine volume, knee reflex and other vital signs were closely observed, and the subject's conditions were evaluated again, 1 time/3 days. The drug was stopped at the end of pregnancy, with 5 days as 1 course.

Uterine artery examination

We adopted color Doppler ultrasonic diagnostic instrument, with the probe frequency of 2.5-5 MHz, transmission power less than 10 mW/cm. Uterine artery S/D (maximum blood flow velocity

during the systolic period/end diastolic blood flow velocity), RI (resistance index), and PI (pulsation index) were measured. The examination include moderate filling bladder, calm breathing, supine position, routine inspection of fetal double parietal diameter, head circumference and femur, humerus length, inspection of amniotic fluid, placenta, judge fetal intrauterine development. Uterine artery examination: The lower uterine muscle wall was found by avoiding intestinal gas interference on both sides of the cervical uterine junction. The intergarterial artery was shown between the pelvic wall and the lower uterine muscle layer, and the uterine artery blood flow signal was found near the cervical opening. Sampling volume was placed on the upper branch of the uterine artery at the lateral edge of the muscle-wall, to obtain the continuous, stable and clutter free images of the uterine artery blood flow spectrum.

Umbilical artery examination

The umbilical artery of the near placenta was relatively stable. The umbilical artery was measured when the fetus was relatively quiet. The included Angle between the pulse Doppler sampling line and the umbilical artery was 60°. The PI, RI and S/D of the fetal umbilical artery were measured after taking the blood flow spectrum of more than 5 consecutive cardiac cycles.

Specimen collection and treatment

After receiving different treatments, 5 mL of peripheral fasting venous blood of the patients was retained, naturally coagulated for 30 min and centrifuged at 4000 RPM for 10 min. The serum was separated and frozen for storage.

The placenta was delivered by cesarean section in both groups. The median tissues of maternal placenta was collected in the size of 2 cm × 2 cm × 2 cm, rinsed and removed the blood, and then placed in the -80 °C refrigerator for storage.

Expression of kallikrein gene

High-purity RNA was extracted from blood and placenta specimen, and cDNA was synthesized by reverse transcription polymerase chain reac-

tion method, with actin β as the internal parameter, kallikrein plasmid as the external parameter, 5'-CACAGCCCAGTTTGTTCAT-3' as forward primer, and 5'-GGAAGGTGGCAAAGACAC-3' as reverse primer. Cycle parameters: Pre-degeneration at 94 °C for 5 min, denatosis at 94 °C for 50 s, 56 °C annealing 1 min, 72 °C extends 50 s, for a total of 35 cycles, and finally 72 °C extends from 10 min to 4 °C to complete the reaction. The products of polymerase chain reaction and plasmid amplification were observed by agarose gel electrophoresis.

The levels of PAPP-A, SPI, PLGF, and HPL in serum and placental fluid were determined by enzyme linked immunosorbent assay (ELISA). Placental tissue was washed twice with 400 mL of TrisHcl (the inhibitor containing amino acid proteases) and centrifuged at 4 °C for 20 min, and then the supernatant was collected. The microplate reader (Biotek, Model: EL \times 800) was operated by specific operation steps strictly according to the product instructions.

The levels of TGF-1, VCAM-1, and E selectin in serum were determined by ELISA. The localization of TGF-1, VCAM-1, and E selectin in placental tissue was detected by immunohistochemical streptomycin antibiotin protein - peroxidase ligating method, and then the protein expression was analyzed semi-quantitatively.

Coagulation function

Before treatment and 5 days after treatment, 6 mL of elbow venous blood was collected from both groups, centrifuged at 3500 rpm for 10 min,

and the supernatant was taken. The coagulation functions, including prothrombin time (PT), activated partial thrombin time (APTT), thrombin time (TT) and fibrinogen (FIB), were detected with the automatic biochemical analyzer.

Statistical methods

The data of detection indexes of the two groups were expressed by $\bar{x} \pm s$, and processed with SPSS 22.0 (IBM Corp., Armonk, NY, USA) statistical analysis software. The differences between different groups were compared with variance analysis or chi-square test, and the data in the same group was tested with *t* test. $P < 0.05$ represents statistically significant difference.

Results

Comparison of general data

Seventy patients with severe PE were enrolled, 35 of them were grouped into the control group, aged 22~36 yr old, with the average age of (26.47 \pm 1.92) yr old, pregnant for 26~38 weeks, with the average gestational age of (35.63 \pm 2.21) weeks. Another 35 patients were grouped into the observation group, pregnant for 26~39 weeks, with the average gestational age of (36.72 \pm 1.97) weeks. All enrolled patients have completed the trials. The differences in age, gestational age and other general data were compared between the observation group and the control group, without statistical significance, which were comparable (Table 1).

Table 1: Comparison of general clinical data between observation and control groups (n=35)

Group	Age (yr)	BMI (kg)	Gestational age (week)	Pregnant times
Control group	29.36 \pm 3.76	65.87 \pm 7.05	35.80 \pm 3.18	1.94 \pm 0.91
Observation group	28.34 \pm 4.10	63.73 \pm 6.34	36.10 \pm 2.53	2.08 \pm 0.89
<i>t</i>	1.078	1.339	0.436	0.667
<i>P</i>	0.620	0.521	0.360	0.973

Comparative analysis of 24 h urinary protein quantification, systolic pressure and diastolic pressure

Before treatment, 24 h urinary protein quantification, systolic pressure and diastolic pressure were compared, without statistical significance. After treatment, 24 h urinary protein quantification, systolic pressure and diastolic pressure in the observation group were significantly better than those in the control group, and the differences were statistically significant ($P < 0.05$), as shown

in Table 2. The 24 h urinary protein quantification, systolic pressure and diastolic pressure of patients treated with LMWH combined with magnesium sulfate was better than those treated with magnesium sulfate, which may be related to the anti-inflammatory effects of LMWH combined with magnesium sulfate, indicating that LMWH combined with magnesium sulfate can effectively reduce vascular tension and inhibit neuromuscle activity.

Table 2: Comparative analysis of 24 h urinary protein quantification, systolic pressure and diastolic pressure in both groups ($\bar{x} \pm s$)

Time	Group	24 h urinary protein quantification	Systolic Pressure	Diastolic Pressure
Before treatment	Control group (n=35)	9.97±3.01	160.15±10.32	97.23±6.42
	Observation group (n=35)	10.32±2.97	160.23±10.25	97.01±6.37
	<i>t</i>	0.695	1.138	0.493
	<i>P</i>	0.254	0.656	0.122
After treatment	Control group (n=35)	7.92±2.04	127.95±8.34	93.25±5.26
	Observation group (n=35)	6.01±1.35	119.64±7.93	87.34±5.11
	<i>t</i>	7.956	3.628	4.223
	<i>P</i>	0.001	0.029	0.015

Changes in uterine artery blood flow

Before treatment, uterine artery S/D of the observation group was 2.56 ± 0.31 , and that of the control group was 2.49 ± 0.29 , without statistical significance. After treatment, the uterine artery S/D in patients with severe PE of the observation group was lower compared with that in the control group, indicating a better treatment effect. Before treatment, RI was 0.63 ± 0.03 and 0.59 ± 0.04 in the patients of the observation group and the control group, respectively, without statistical significance. After treatment, RI significantly decreased in the observation group. And before treatment, uterine artery PI in patients with severe PE of the observation group was 1.53 ± 0.09 , which was significantly lower compared with that before treatment, as shown in Fig. 1.

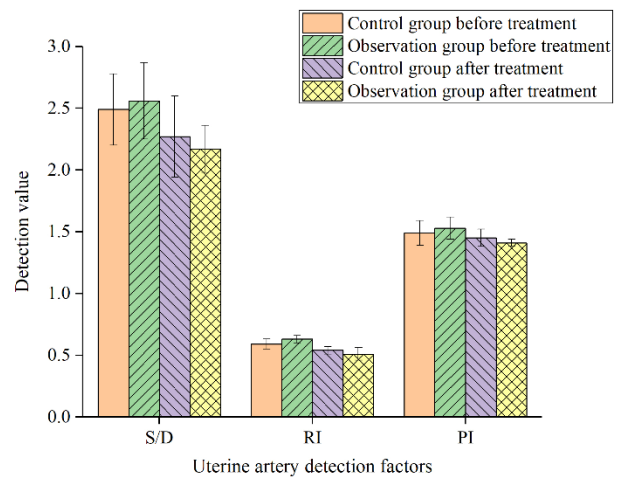


Fig. 1: Changes in uterine artery blood flow

Changes in umbilical artery blood flow

Before treatment, there were no statistically significant difference in S/D, RI, and PI of umbilical artery, which were also not statistically significant after treatment, as shown in Fig. 2.

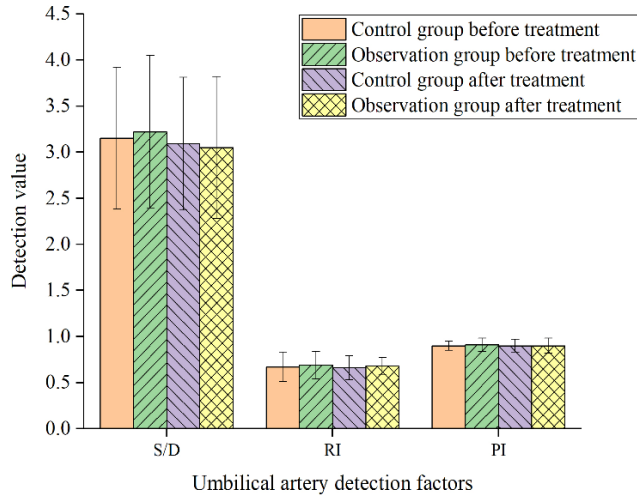


Fig. 2: Changes in umbilical artery blood flow

Umbilical artery blood flow S/D reflects placental microcirculation impedance and irrigation flow of placental blood flow, which can increase blood coagulation in pregnant women with severe PE, resulting in increased placental blood vessel resistance, decreased fetal placental blood circulation, increased S/D, leading to intrauterine hypoxia and limited growth. All the RI, PI and S/D values after treatment were significantly lower than those before treatment in the observation group, which was significantly lower than that of the control group, while there was no significant difference in RI, PI and S/D values be-

fore and after treatment in the control group. The combined treatment could effectively improve placental microcirculation in patients and help to correct the fetal growth restriction.

Expression of kallikrein

Before treatment, the kininase level in the blood of the control group and the observation group were 0.85 ± 0.12 , 0.83 ± 0.08 , respectively, without statistically significant difference. However, after treatment, kininase expression in serum of the observation group (1.47 ± 0.21) was higher than that of the control group (0.87 ± 0.09), with statistically significant difference ($P < 0.05$).

Levels of PAPP-A, SPI, PLGF, and HPL

Levels of PAPP-A, SPI, PLGF and HPL in patients of the two groups were tested with rank-sum test. The level of PAPP-A in serum and placenta tissue of the observation group after treatment was (1.21 ± 0.42), which was lower than those in the control group (2.03 ± 0.67), with statistical significance ($P = 0.006$). After treatment, the levels of PLGF and HPL in serum and placental tissue of the observation group were also lower than those of the control group, with no statistical significance between the two groups. After treatment, the level of SPI in serum and placental tissue of the observation group was (0.92 ± 0.09), which was higher than that of the control group (0.83 ± 0.07), with no statistical significance between the two groups (Table 3 and Table 4).

Table 3: Comparison of levels of PAPP-A, SPI, PLGF, and HPL in the two groups ($\bar{x} \pm s$)

Group	PAPP-A (mIU / mL)	SPI ($\mu\text{g}/\text{mL}$)	PLGF (pg/mL)	HPL ($\mu\text{g}/\text{mL}$)
Control group (n=35)	2.03 ± 0.67	0.83 ± 0.07	334.95 ± 45.87	15.93 ± 1.73
Observation group (n=35)	1.21 ± 0.42	0.92 ± 0.09	326.14 ± 45.42	15.40 ± 1.72
t	5.194	0.887	1.150	1.053
P	0.006	0.221	0.995	0.626

Table 4: Comparison of levels of PAPP-A, SPI, PLGF, and HPL in the two groups ($\bar{x} \pm s$)

<i>Group</i>	<i>PAPP-A</i> (<i>mIU / mL</i>)	<i>SPI</i> ($\mu\text{g/mL}$)	<i>PLGF</i> ($\mu\text{g/mL}$)	<i>HPL</i> ($\mu\text{g/mL}$)
Control group (n=35)	1.91±0.63	0.85±0.05	371.16±45.86	15.84±1.69
Observation group (n=35)	1.19±0.47	0.92±0.08	349.42±45.21	16.01±1.73
<i>t</i>	2.226	0.882	0.408	0.654
<i>P</i>	0.014	0.551	0.152	0.814

Levels of TGF-1, VCAM-1, and E selectin

Before treatment, the differences of TGF- β 1, VCAM-1 and E selectin in serum and placental tissue were not statistically significant, which was comparable. After treatment, the levels of TGF- β 1, VCAM-1 and E selectin in the serum in the control group were 179.45±23.14, 103.26±13.64 and 62.24±2.43, respectively, and those of the observation group were 257.37±40.12, 45.28±4.23 and 44.57±3.86, respectively. After treatment, the levels of TGF- β 1 in serum and placental tissue of the observation group were higher than those of the control group, while the levels of VCAM-1 and E selectin were lower than those of the control group, with statistical significance ($P < 0.05$).

Comparative analysis of the coagulation function indexes in the two groups

After treatment, the levels of PT, APTT, and TT of the two groups were higher than those before

treatment, FIB level was lower than that before treatment. The levels of PT, APTT, and TT of the observation group were higher than that of control group, and FIB level was lower than that of the control group ($P < 0.05$), as shown in Table 5. Four coagulation indexes are commonly used for clinical monitoring of blood coagulation status and anticoagulant dosage. In the observation group and control group before treatment, PT was shorter than normal level and FIB was lower than normal level, indicating that the coagulation mechanism was abnormal and the blood was in a state of hypercoagulation. After treatment, PT and FIB in the observation group were significantly increased and higher than those in the control group, while there was no significant change in the control group, indicating that low molecular weight heparin combined with magnesium sulfate can effectively improve the hemagglutination status of pregnant women.

Table 5: Comparison of coagulation function indexes between control and observation groups ($\bar{x} \pm s$)

<i>Time</i>	<i>Group</i>	<i>PT (s)</i>	<i>APTT (s)</i>	<i>TT (s)</i>	<i>FIB (g / L)</i>
Before treatment	Control group (n=35)	8.60±0.79	27.31±2.04	11.48±1.42	5.28±0.54
	Observation group (n=35)	8.53±0.56	27.37±1.94	11.57±1.32	5.34±0.52
	<i>P</i>	0.25	0.21	0.16	0.34
After treatment	Control group (n=35)	11.97±1.06	30.97±1.02	13.36±1.04	3.26±0.21
	Observation group (n=35)	14.21±0.46	35.02±0.98	15.01±0.92	2.15±0.17
	<i>P</i>	0.000	0.000	0.000	0.000

Discussion

Severe PE is a relatively common pregnancy disease in obstetrics and gynecology, with its incidence rate about 3.9%. In the past, relevant scholars took more immediate pregnancy termination methods in patients with severe PE, regardless of the number of weeks of pregnancy, resulting in an increased rate of iatrogenic preterm birth and perinatal mortality. In recent years, with the continuous development of intensive medicine, more and more obstetricians choose to adopt conservative treatment to extend the time of maternal pregnancy to improve neonatal survival while ensuring the safety of maternal life. In the past, simple magnesium sulfate was treated clinically to prolong the pregnancy of PE (22).

Although magnesium sulfate can prolong the gestational age and promote fetal development, the protective effect on the patient's liver and kidney function is not obvious, and it cannot improve the high coagulation state of blood (23). LMWH is a small molecular fragment product of ordinary heparin demossynthesis and reduces the inhibition of thrombin (24). LMWH combined with magnesium sulfate has many advantages compared with simple magnesium sulfate to quickly improve the clinical symptoms (25).

In this study, the observation group had significantly better efficiency than those in the control group, indicating that magnesium sulfate with LMWH can effectively improve clinical effects and improve the viability of the fetus. In this study, 24 h urinary protein quantification and diastolic pressure in patients treated with magnesium sulfate and LMWH were significantly better than those treated with simple magnesium sulfate, which may be related to the anti-inflammatory effects of LMWH combined with magnesium sulfate, indicating that LMWH combined with magnesium sulfate can effectively reduce vascular tension and inhibit neuromuscle activity. In this study, the coagulation function indexes were significantly better than that in patients with simple magnesium sulfate treatment,

which may be related to spasmodic, sedation and blood reduction in LMWH combined with magnesium sulfate, indicating that LMWH combined with magnesium sulfate can effectively improve the coagulation function of patients.

Conclusion

The use of LMWH combined with magnesium sulfate for severe PE, effectively control the disease, can effectively improve the pregnant blood hypercoagulation, reduce the systolic pressure, improve placental microcirculation, help to promote fetal growth, improve efficacy, and high safety, has clinical promotion and application value.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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