

Original Research Article

Efficacy of normodyne-magnesium sulfate combination treatment on pregnancy-induced hypertension, and its effect on VEGF and Flt-1 levels

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Abstract

Purpose: To investigate the efficacy of the combined use of normodyne and magnesium sulfate in the treatment of pregnancy-induced hypertension, and its effect on vascular endothelial growth factor (VEGF) and factor receptor-1 (Flt-1) levels in serum.

Methods: A total of 100 patients with pregnancy-induced hypertension attending Maternal and Child Health Hospital of Xinjiang Uygur Autonomous Region, Xinjiang, China, were categorized as Group A, and then further subdivided into control sub-group (who were treated with magnesium sulfate only) and study sub-group (treated with magnesium sulfate plus normodyne). Furthermore, 100 healthy pregnant women attending the hospital for prenatal examination during the same period were categorized as Group B. Serum expressions of VEGF and Flt-1 in all patients were determined and compared. The therapeutic effect, adverse reactions, adverse pregnancy outcomes, blood pressure before and after treatment, 24 h proteinuria, and serum expression levels of VEGF and Flt-1 in the study and control groups were determined and compared.

Results: Serum VEGF levels in patients with pregnancy-mediated hypertension were significantly lower than those of healthy pregnant women, and Flt-1 was raised in healthy pregnant women ($p < 0.05$). In the study group, treatment was markedly more effective, and the degree of amelioration of blood pressure, 24 h proteinuria, serum VEGF, and Flt-1 were significantly higher than for control sub-group. There were lower adverse pregnancy outcomes in study sub-group than in control ($p < 0.05$).

Conclusion: The combination of magnesium sulfate and normodyne produces greater clinical efficacy in the treatment of patients with pregnancy-induced hypertension than magnesium sulfate alone, and also shows a high safety profile.

Keywords: Normodyne, Magnesium sulfate, Pregnancy-induced hypertension, VEGF, Flt-1

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INTRODUCTION

Pregnancy-induced hypertension (PIH) refers to abnormal blood pressure in pregnant women caused by systemic vasospasm. Severe

conditions may result in abortion, which poses a serious threat to the lives of pregnant women and fetuses [1]. Currently, drug therapy is the mainstay of the treatment of PIH so as to control and ameliorate the blood pressure of patients.

However, given the potential side effects on the fetus, the selection of drugs for treatment remains one of the most pressing issues to be addressed [2]. Magnesium sulfate is currently the main drug in the clinical treatment of PIH. It controls the concentration of acetylcholine in blood vessels and improves the maternal hemoglobin concentration by antagonizing calcium ions and magnesium ions, in order to further relieve the vasospasm [3]. However, current clinical data have revealed an unsatisfactory curative effect of single-drug therapy for PIH, thereby necessitating the use of combination drug therapy [4]. Normodyne, a receptor blocker, reduces the load on the heart by expanding peripheral resistance in vessels, and it also slows the heart rate. It has demonstrated great potential in the treatment of PIH [5]. As a vascular inducer, VEGF regulates the development and repair of blood vessels, and its expression level can also reflect the endothelial function of blood vessels [6]. Factor receptor-1 (Flt-1) protein, a complex kinase receptor, combines with VEGF and inhibits the function of VEGF. Excessive expression of Flt-1 protein affects the permeability and integrity of the vascular wall. Research has found that an increase in Flt-1 expression may be one of the risk factors for adverse pregnancy outcomes [7]. Notwithstanding some investigations on the combined use of Normodyne and magnesium sulfate in the treatment of PIH [8], there is no research on the expressions of VEGF and Flt-1 in sera of patients with PIH. The present investigation was designed to study the effect of combined use of Normodyne and magnesium sulfate in patients with PIH, and the effect of treatment on serum levels of vascular endothelial growth factor (VEGF) and factor receptor-1 (Flt-1).

EXPERIMENTAL

General data on patients

One hundred PIH subjects on admission from June 2016 to July 2018 at the Maternal and Child Health Hospital of Xinjiang Uygur Autonomous Region, were prospectively selected as Group A. The mean age of the patients was 28.71 ± 2.63 years. Magnesium sulfate was used for treating 50 of the patients (control sub-group), while the rest 50 (study sub-group) received magnesium sulfate plus normodyne. In addition, 100 healthy pregnant women who underwent a prenatal examination in this same hospital during the same period were selected and categorized as Group B. The mean age of all included participants was 28.56 ± 2.55 years.

Inclusion and exclusion criteria

Patients who met the clinical diagnostic criteria related to pregnancy-induced hypertension were included [9]. Those with severe organ dysfunction and hereditary diseases; patients with other pregnancy complications, and patients with poor compliance, were excluded. All subjects and their family members consented willingly to take part in the study, and they signed informed consent forms. This study received approval from the Hospital Ethics Committee of Maternal and Child Health Hospital of Xinjiang Uygur Autonomous Region (approved no. 2016-FD2453). This study was carried out in full accordance with the Declaration of Helsinki [10].

Treatments

Control subjects were given magnesium sulfate only: 20 g magnesium sulfate was diluted in 1000 mL of 5 % glucose and used for intravenous drip treatment, at a drip rate of 1.5 g/h, once a day. Those in study group received magnesium sulfate plus normodyne: 20 g magnesium sulfate was diluted in 1000 mL of 5 % glucose and given by intravenous drip, at a drip rate of 1.5 g/h, and 100 mg normodyne was diluted in 5 % glucose injection or 0.9 % sodium chloride to 250 mL and given by intravenous drip, at an intravenous drip rate of 2 mg/min. The curative effect was analyzed after 1 week of treatment.

Treatment outcomes

Therapeutic effect

The treatment effectiveness in the two groups were determined. The patients were divided into markedly effective (normal blood pressure and disappearance of symptoms and signs); effective (amelioration of blood pressure, symptoms, and signs by 50% or more), and ineffective (no obvious amelioration of blood pressure, symptoms and signs). Treatment effectiveness (TE) was calculated as shown in Eq 1.

$$TE (\%) = \{(ME + E)/N\}100 \dots\dots\dots (1)$$

where ME and E are the number of patients in whom treatment was markedly effective respectively, while N is the total number of patients.

Blood pressure and proteinuria

Blood pressure was monitored daily while the 24 h proteinuria before and after treatment was obtained by urinary protein precipitation.

Degree of proteinuria was classified as *mild proteinuria* (urinary protein content < 0.5 g/24h; *moderate proteinuria* (urinary protein contents of 0.5 - 2.0 g/24 h; *severe proteinuria* (urinary protein > 2.0 g/24 h).

Serum levels of VEGF and Flt-1

ELISA was used to detect and compare the expressions of VEGF and Flt-1 in the serum of the two groups before and after treatment. Fasting venous blood (3 mL) was drawn from each subject, centrifuged at 3000 g for 10 min, and the serum was isolated in polyethylene tubes and stored at -28 °C. All specimens collected were thawed overnight at 4-8°C, centrifuged, and then VEGF and Flt-1 assay kits were used to determine VEGF and Flt-1 in a fully automated enzyme assay system. The operation steps strictly complied with the kit instructions.

Adverse reactions

The adverse reactions which included nausea, vomiting, and dizziness, were recorded and compared. Pregnancy outcomes in the two groups were recorded and compared.

Statistics

Data were processed using SPSS 19.0 software, while graphics were plotted with GraphPad Prism 6. The counting data are expressed as rate and analyzed using χ^2 . Measurement data are presented as mean \pm SD. Two-group comparison was done with the independent-sample *t*-test, while paired *t*-test was employed for comparing

pre- and post-treatment data. Values of $p < 0.05$ indicated statistically significant differences.

RESULTS

Patients

Table 1 shows that data on age, gestational weeks, and BMI before pregnancy were comparable among the three groups of subjects ($p > 0.05$).

Expressions of VEGF and Flt-1 in pregnant ladies with PIH and healthy pregnant controls

It was found that the expression of VEGF in patients with PIH was significantly lower than that in healthy pregnant women, and the expression of Flt-1 in patients with PIH was markedly up-regulated, relative to that in healthy pregnant women (Figure 1).

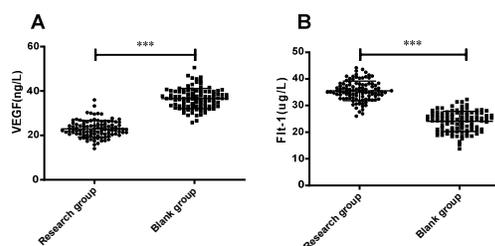


Figure 1: Expression of VEGF and Flt-1 in pregnant ladies with PIH and normal pregnant ladies. A: VEGF expression was lower in research subjects than in blank subjects; B: Flt-1 was highly expressed in the research subjects, relative to the blank subjects. *** $P < 0.001$

Table 1: General profile of patients

Variable	Control (n=50)	Study (n=50)	Blank (n=100)	F/ χ^2	P-value
Age (years)	28.56 \pm 2.54	28.69 \pm 2.71	28.59 \pm 2.67	0.036	0.965
BMI before pregnancy (kg/m ²)	22.33 \pm 2.24	22.62 \pm 2.19	22.54 \pm 2.26		
Educational level				0.021	0.990
Below Junior high school	10(20.00)	11(20.75)	21(21.00)		
Junior high school or above	40(80.00)	42(79.25)	79(79.00)		
Residence				0.203	0.904
Rural	19(38.00)	22(96.61)	38(38.00)		
Urban	31(62.00)	31(3.39)	62(62.00)		
Birth history				0.494	0.781
Primipara	37(74.00)	39(73.58)	78(78.00)		
Multipara	13(26.00)	14(26.42)	22(22.00)		
Gestational week	27.21 \pm 2.33	27.34 \pm 2.25	27.27 \pm 2.29	0.042	0.959

Therapeutic effectiveness

Table 2 reveals that the control group had 15 cases of markedly effective, 20 cases of effective, and 15 cases of ineffective, with treatment effectiveness of 70 %. The study group had 27 cases of markedly effective, 22 cases of effective, and 4 cases of ineffective, with treatment effectiveness of 92.45 %. The treatment effectiveness was markedly higher in the study subjects than in control subjects.

24-h proteinuria and blood pressure

There was no significant difference in blood pressure and 24 h proteinuria between the 2 groups before treatment. However, blood pressure and 24 h proteinuria of the 2 groups were markedly decreased after treatment. The study group had a better outcome in terms of the 2 parameters after treatment, when compared to the control subjects. These results are presented in Figure 2.

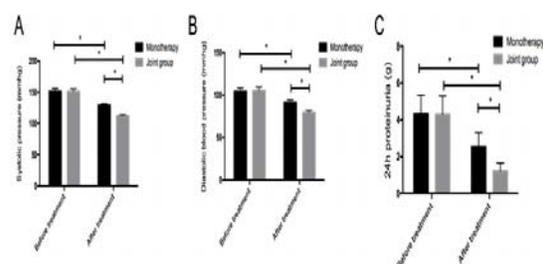


Figure 2: Comparison of pre- and post-treatment blood pressure and 24-h proteinuria values between the two groups. A: comparison of systolic blood pressure between the two groups before and after treatment; B: comparison of diastolic blood pressure between the two groups pre- and post-treatment; C: comparison of 24-h proteinuria between the two groups before and after treatment. * $P < 0.05$

Table 2: Comparative therapeutic effectiveness

Curative effect	Control group n=50	Study group n=50	χ^2	P-value
Markedly effective	15 (30.00)	27 (50.94)	4.672	0.031
Effective	20 (40.00)	22 (41.51)	0.164	0.685
Ineffective	15 (30.00)	4 (7.55)	8.622	0.003
Total effectiveness	35 (70.00)	49 (92.45)	8.622	0.003

Table 3: Incidence of adverse events {n (%)}

Symptom	Control (n=50)	Study (n=50)	χ^2	P-value
Nausea	2 (4.00)	3 (5.66)	0.154	0.695
Vomiting	2 (4.00)	3 (5.66)	0.154	0.695
Dizzy	3 (6.00)	3 (5.66)	0.005	0.941
Total incidence	7 (14.00)	9 (16.98)	0.174	0.676

Serum VEGF and Flt-1 expressions

The serum expression levels of VEGF and Flt-1 in the two groups were comparable before treatment ($p > 0.05$). However, post-treatment, both groups had markedly up-regulated expressions of serum VEGF, and markedly decreased expression level of Flt-1 ($p < 0.05$), with a higher level of VEGF and a lower level of Flt-1 observed in study subjects. These data are presented in Figure 3.

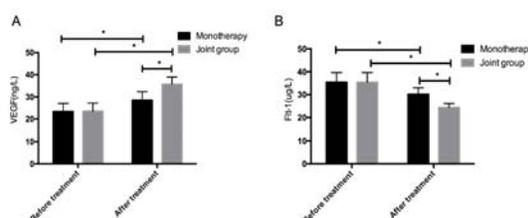


Figure 3: Serum VEGF and Flt-1 expressions before and after treatment. A: serum VEGF levels before and after treatment; B: Comparison of Flt-1 before and after treatment

Adverse events

Table 3 shows that the major adverse events recorded and compared between the 2 groups of subjects during treatment were dizziness, nausea and vomiting. The incidence of adverse events were comparable between the 2 groups.

Comparison of pregnancy outcomes

It was found that there were 2 cases of fetal growth restriction and stillbirth, 3 cases of fetal growth restriction and neonatal death, 3 cases of fetal growth restriction and neonatal low birth weight, and 3 cases of neonatal low birth weight in control subjects, accounting for 22 % (11/50) cases of adverse pregnancy outcomes (11/50).

Table 4: Pregnancy results in the 2 groups of patients {n (%)}

Variable	Control group n=50	Study group n=50	χ^2	P-value
Fetal growth restriction and stillbirth	2(4.00)	1(1.89)	0.406	0.524
Fetal growth restriction and neonatal death	3(6.00)	1(1.89)	1.166	0.280
Fetal growth restriction and low birth weight	3(6.00)	1(1.89)	1.166	0.280
Neonatal low birth weight	3(6.00)	0	3.275	0.070
Total incidence	11(22.00)	3(5.66)	5.849	0.016

One case of fetal growth restriction and stillbirth, 1 case of fetal growth restriction and neonatal death, and 1 case of neonatal low birth weight were seen in study subjects, accounting for perinatal adverse pregnancy incidence of 5.66%. These results are presented in Table 4.

Serum VEGF and Flt-1 expression in patients with different pregnancy outcomes

Patients with PIH were divided into normal pregnancy group (89) and adverse pregnancy group (14) according to different pregnancy outcomes. The serum expressions of VEGF and Flt-1 in patients with different pregnancy outcomes were compared. The results showed that the serum VEGF expression was markedly higher in the normal pregnancy patients than in adverse pregnancy patients, while Flt-1 expression was markedly decreased in adverse pregnancy patients (Figure 4).

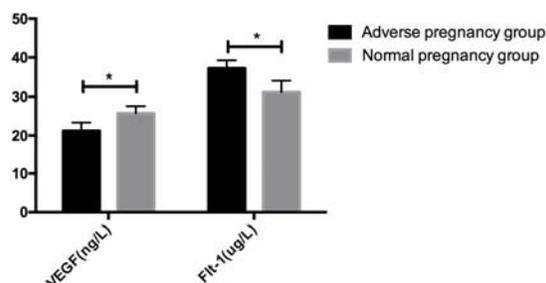


Figure 4: Serum VEGF and Flt-1 expressions in patients with different pregnancy outcomes; * $p < 0.05$

DISCUSSION

Pregnancy-induced hypertension (PIH) is a common complication of pregnancy. With improvements in people's living standards, the intake of high-fat and high-salt diet have led to a higher incidence of PIH, which endangers the lives and health of pregnant women and fetuses [11]. The clinical symptoms of PIH are rather hidden before 20 weeks of pregnancy. This frequently results in varying degrees of damage to the mother and the fetus at the time of diagnosis [12]. Therefore, for patients with PIH, timely diagnosis and treatment are of great clinical significance.

Previous research [13] has revealed a relationship between VEGF and the occurrence of PIH. Specifically, VEGF regulates the growth and development of the placenta and uterine blood flow by promoting vascular permeability. Moreover, Flt-1 combines with free VEGF in blood circulation thereby inhibiting the synthesis of trophoblast DNA [14]. In this study, results showed that serum VEGF level was significantly lower in PIH subjects than in healthy pregnant women, and Flt-1 expression was significantly higher than that of healthy pregnant women, which is suggestive of the participation of VEGF and Flt-1 in the pathogenesis of PIH. Previous studies have indicated that when the expression of VEGF is reduced, it may affect the proliferation and differentiation of trophoblast cells, as well as the endothelial function of blood vessels, thereby affecting the sensitivity of vasopressors and the contractile function of blood vessels, leading to hypertension. When Flt-1 specifically binds to VEGF, the decrease in VEGF is closely related to the increase in Flt-1 [15,16]. This explains the results of the present study.

Magnesium sulfate and normodyne are commonly used antihypertensive drugs in antenatal clinics. Magnesium sulfate reduces blood pressure and increases the blood flow in blood vessels. Normodyne, a salicylamide derivative, selectively blocks α_1 -receptor and non-selectively blocks β -receptor. It inhibits renin secretion, reduces peripheral vascular resistance, and promotes blood pressure reduction. Moreover, it dilates blood vessels and reduces blood pressure by inhibiting sympathetic nerve excitation and reducing the secretion of theophenamine, with high safety profile [17]. The treatment outcome of combined use of the two drugs was significantly better than that of magnesium sulfate alone.

This study also found that when the two drugs were used together, the serum concentrations of VEGF and Flt-1 in PIH subjects were more effectively improved. Furthermore, the combinational use of the drugs resulted in a more favorable outcome in terms of the 24-h proteinuria and blood pressure than that of magnesium sulfate alone. Subsequently, the clinical adverse reactions of different groups

were also compared. The results revealed that both groups did not differ markedly in adverse events, which suggested that the combined medication had a high safety profile and did not increase the discomfort of patients.

The incidence of adverse pregnancy was markedly lower in subjects treated with combined drugs than in patients treated with single drug. In all, these results indicate that the combination of magnesium sulfate and Normodyne produced a higher curative effect for patients with pregnancy-induced hypertension. In addition, the results showed that serum VEGF in subjects with adverse pregnancy outcomes was significantly lower than that of patients with normal pregnancy, while Flt-1 was significantly higher in patients with normal pregnancy. Previous research [18] found that the combined use of magnesium sulfate with other drugs had a higher curative effect on pregnancy-induced hypertension than magnesium sulfate alone, which finding is similar to the results of the present study.

CONCLUSION

The combined use of magnesium sulfate and normodyne exerts a better regulatory effect on the expression of VEGF and Flt-1 in serum of patients with PIH than magnesium sulfate alone. Moreover, the combination drug therapy yields better clinical efficacy and higher safety for patients with PIH. The results obtained only showed that the condition of patients with PIH improves within one week after treatment, which may affect the study results. Therefore, the patients in this study will be further followed up in future studies based on relevant data to support the results of the research.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaoyan Diao designed the study and drafted the manuscript. Xuemei Ma was responsible for the collection and analysis of the experimental data. Xiaoyan Diao and Xuemei Ma revised the manuscript. Both authors read and approved the final manuscript.

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