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Effect of magnesium sulfate on middle cerebral, umbilical artery and uterine arteries Doppler parameters in pregnancy up to 32 weeks

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Introduction: **Effect of Magnesium sulfate (MgSO4) on cerebral vessels are still controversial and it is unclear whether it reduces cerebrovascular events. Studies in this area are still ongoing. The aim of this study was to assess the effect of MgSO4 on fetal middle cerebral artery, umbilical artery and uterine artery using Doppler parameters in pregnancy up to 32 weeks.** ***Patients and Methods:*** **This was a quasi-experimental investigation in which we evaluated pregnant women up to 32 gestational weeks who were admitted in Imam Khomeini Hospital, Tehran University of Medical Sciences between April 2016 and May 2017. Participants were admitted and after baseline evaluations, they were entered in the study. Before and after administration of Magnesium sulfate Doppler evaluation of uterine (UA), umbilical (UMA) and middle cerebral artery (MCA) performed for pulsatility index (PI), resistance index (RI) and systole/diastole ratio (S/D). The results were compared by paired T-test and Wilcoxon test.** ***Results:*** **Finally, 70 patients enrolled in this study. The mean age of participants was 32.69± 6.01 years. The mean gestational age was 29.84± 1.56 weeks. Umbilical artery pulsatility index (UPI) was significantly decreased after MgSO4 injection (P= 0.001), umbilical resistance index in umbilical artery (URI) and S/D also were significantly decreased (P<0.0001). In fetal MCA Doppler, middle cerebral artery pulsatility index (MPI) (P<0.0001) and middle cerebral artery resistance index (MRI) (P<0.0001) were increased significantly after MgSO4 injection and peak systolic velocity (PSV) had not changed significantly. In uterine artery Doppler, uterine artery pulsatility index (UTPI) and uterine artery resistance index (UTRI) were significantly decreased after drug injection (P<0.0001 in both). We showed that there was a significant difference between MPI/UPI ratio before and after administration of maternal MgSO4 (0.73± 0.31 vs. 0.64± 0.24; P<0.0001).** ***Conclusion:*** **Intravenous administration of magnesium sulfate in pregnant women up to 32 gestational weeks resulted in a decrease in PI and RI in umbilical artery and uterine artery, and increased in MCA Doppler indices.**

INTRODUCTION

Magnesium sulfate (MgSO4) can help prevent serious complications in severe preeclampsia (1, 2). Effect of MgSO4 on cerebral vessels are still controversial and it is unclear whether it reduces cerebrovascular events. Studies in this area are still ongoing (2, 3). It reduces the risk of eclampsia in antepartum and postpartum women, irrespective of the severity of preeclampsia (4). The mechanism of action may include changes in vascular permeability and reduction of edema (1). It seems to have no adverse effects on the fetus (4), but in response to MgSO4 injection, fetal brain perfusion may also alter (5). It protects against neonatal cerebral hemorrhage, decreases neuronal damage following brain injury and improves survival in extremely low-birthweight infants (6, 7). Some studies have found that magnesium sulfate does not have a major impact on disease progression in mild preeclampsia women (8, 9). These findings have raised questions about the routine use of magnesium sulfate prophylaxis in mild preeclampsia. Thus, it is essential to critically analyze the effect of prophylactic magnesium sulfate on the fetus (10).

In pregnancy, usually umbilical artery is tested by Doppler ultrasound but some recent studies also test maternal and fetal middle cerebral arteries (MCA) (11-14). It is shown that abnormal umbilical artery waveform is a strong and independent predictor of fetal outcome (15). In one study, the reference value of resistive index (RI) and pulsatility index (PI) have been investigated in Iranian patients (14). The

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ratio of middle cerebro-placental ratio (C/P ratio) can be a good indicator of fetal prognosis and fetal well-being (12, 14). Doppler ultrasound is a useful tool for studying pathophysiological mechanisms that can affect the fetal hemodynamic status (16, 17). Assessing the changes in the arteries with Doppler ultrasound can show the adaptation of fetus with this situation (18). An increase in umbilical artery resistance shows as a decrease in perfusion. If the situation continues, RI in the middle cerebral artery will decrease (19). Investigating the mechanism of action of MgSO4 on the fetus is one of the important issues in this disease (20).

The aim of this study was to assess the effect of MgSO4 on fetal middle cerebral artery, umbilical artery and uterine artery by using Doppler parameters in pregnancy up to 32 weeks.

METHODS

This was a quasi-experimental investigation in which we evaluated pregnant women up to 32 gestational weeks who were admitted in the Imam Khomeini University Hospital, Tehran University of Medical Sciences, between April 2016 and May 2017.

Eligibility

We recruited all pregnant women who were candidate to received MgSO4 for any indication like pre-eclampsia prevention or neuro-protection and pregnancy termination was done less than 12 hours. Fetal gestational age up to 32 weeks (confirmed by last menstrual period and first trimester ultrasonography). Exclusion criteria were multiple pregnancy, congenital fetal abnormality, myasthenia gravis, chronic renal failure and Less than 12 hours before magnesium sulfate administration, pregnancy was terminated due to each cause.

Study design

Eligible subjects were requested to fulfill informed consent to participate in the study. After routine examination in the clinic, they were admitted in the ward and further evaluations were performed. They received routine care for pregnant women and necessary treatments. After baseline evaluations, they were entered in a before-after study. Firstly, they underwent Doppler evaluation of uterine and fetal vessels performed by a perinatologist fellowship with the Philips Ultrasound System (Philips Affiniti 50 Ultrasound, Bothell, WA) using a 5-MHz sector transducer. It was done with spatial peak temporal average intensities below 50 mW/cm2 and a high pass filter at 50–100 Hz. Measurements were obtained from the umbilical artery in free loop of umbilical cord at the midsection of umbilical cord and the proximal portion of middle cerebral artery. Uterine artery measurement was performed in inguinal and in the site of separating from internal iliac artery. Umbilical artery pulsatility index and fetal middle cerebral artery pulsatility index were measured during a period without fetal breathing. Resistance index (RI), pulsatility index (PI) and systole/diastole ratio was measure for each artery. Subsequently, after 12 hours they received magnesium sulfate in prophylactic doses of 6 g intravenously diluted in 12 mL normal saline given slowly over 30 minutes and 2 g/hours for 12 hours intravenously. After receiving MgSO4, patients again underwent Doppler ultrasonography and all of mentioned parameters were measured again.

Ethics

All patients were obtained an informed consent for participation. All patients were informed about the details of study and filled the informed consent form. They allowed by it to publish their data in unnamed format. They were free to exit the study in each step. This study was approved by ethical committee of Tehran University of Medical Sciences and received ethical code as IR.TUMS.IKHC.REC.1397.097.

Statistics

All data were analyzed by SPSS software version 16. Data were checked for normality distribution by Kolmogorov Smirnov test. Paired T-Test and Wilcoxon test were used for analysis of variables between two groups. P Value lesser than 0.05 considered as significant.

RESULTS

Seventy patients were enrolled in the study. The mean age of participants was 32.69± 6.01 years old (minimum: 18 and maximum: 45). The mean gestational age was 29.84± 1.56 weeks (minimum: 25 and maximum: 32 weeks). Other baseline characteristics are listed in table 1.

Before receiving MgSO4 injection, Doppler ultrasonography of umbilical artery showed that 21 subjects have absent diastolic flow (30%) and in four of them, it was reverse (5.7%). This frequency was changed to 18 subjects (25.7%) absent and two subjects reverse (2.9%) after MgSO4 injection. umbilical artery pulsatility index (UPI) was significantly decreased after MgSO4 injection (P= 0.001), umbilical resistance index in umbilical artery (URI) and systole/diastole (S/D) also were significantly decreased (P<0.0001). In fetal MCA Doppler, middle cerebral artery pulsatility index (MPI) (P<0.0001) and middle cerebral artery resistance index (MRI) (P<0.0001) were increased significantly after MgSO4 injection and peak systolic velocity (PSV) had not changed significantly. In uterine artery Doppler, uterine artery pulsatility index (UTPI) and uterine artery resistance index (UTRI) were significantly decreased after drug injection (P<0.0001 in both). Mother heart rate and fetal heart rate also were markedly decreased after drug injection (P=0.025 and P<0.0001, respectively), (Table 2).

Study showed that there was a significant difference between MPI/UPI ratio before and after administration of maternal MgSO4 (0.73± 0.31 vs. 0.64± 0.24; P<0.0001), (Figure 1).

DISCUSSION

Fetal distress caused by hypoxia is one of the effects of preeclampsia on the fetus. Probably, circulation disorder is the most important cause of hypoxia and distress (21). Inadequate placental perfusion can cause fetal reduction of oxygen supply, and thus lead to fetal distress (16, 22). In this study, we assessed the effect of MgSO4 on fetal middle cerebral artery, umbilical artery and uterine artery by using Doppler parameters. The results of our study suggests that prophylactic magnesium sulfate administered to mothers antenatally with different indication admitted up to 32 gestational weeks leads to a reduction in pulsatility index of the umbilical artery and improves placental blood flow. Umbilical pulsatility indices were reduced and middle cerebral artery Doppler indices increased when before-after comparisons were made. When circulation to fetus decreased, firstly changes in S/D, RI and PI in umbilical artery was occurred. The study demonstrated that administration of MgSO4 could lead to decrease the rate of absent and reverse results in diastolic flow umbilical artery ultrasonography findings, which was not statistically significant.

In women with hypertensive disorder in pregnancy, placental perfusion is compromised (23). Decrease in placental perfusion is due to an imbalance in prostacyclin and thromboxane A2 level, altered production of nitric oxide and thickening of the vessel wall due to atheromatous deposition, which leads to decreased luminal flow (23).

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There is also a reduction in the number of terminal villous arteries where placental circulatory readjustments occur (24). Tarzamni et al. showed that the incidence of abnormal umbilical artery pulsatility index in both severe and mild preeclampsia is higher than in normotensive pregnant women (14). In our study, we revealed that maternal systolic blood pressure was significantly decreased after MgSO4 administration (121± 21.86 mmHg vs. 116.14±17.07 mmHg, P<0.001). Although this difference was statistically significant, clinically was not markedly. We also showed that fetal heart rate (157.31± 14.21 vs. 146.18± 1.43) and maternal heart rate (85.90± 6.6 vs. 85.21± 5.61) were significantly decreases after MgSO4 administration (P<0.05). However, control of blood pressure after MgSO4 administration should be considered in every situation. Dasgupta et al. (10) evaluated whether prophylactic magnesium sulfate given in women with mild preeclampsia or gestational hypertension brings any significant change in umbilical artery and fetal middle cerebral artery pulsatility index. They showed that in their study that, there was a significant reduction in the post-magnesium sulfate umbilical artery pulsatility index in the intervention group (median 0.88 [0.82-1.03]) when compared to the placebo group (median 1.00 [0.89–1.10]) and Post-magnesium sulfate fetal middle cerebral artery pulsatility index in the intervention group (median 1.78 [1.63–1.98]) did not show a significant change compared to the placebo group (median 1.65 [1.42–1.91]) (10). Belfort et al. (25) in another study evaluated the effects of magnesium sulfate on maternal and fetal blood flow in pregnancy-induced hypertension. They demonstrated that that a 6-gram loading dose of magnesium sulfate significantly vasodilates the vascular bed distal to the maternal middle cerebral artery, and increases blood velocity in this distribution. There was no significant change in pulsatility index or blood velocity in the central large vessels of the head and neck. There were no acute effects noted in the fetal or placental vessels evaluated (25). In another investigation, Twickler et al. (26) performed a study to estimate the effects of maternal administration of magnesium sulfate on the fetal middle cerebral artery using Doppler. They revealed that in 38 studied fetuses (18 received magnesium sulfate and 20 received placebo) peak systolic velocity was significantly related to gestational age. There were no differences between the study groups for middle cerebral artery peak systolic velocity, average velocity, vessel diameter, or calculated volume flow. However, fetal heart rate significantly decreased after treatment with magnesium sulfate (26). Maged et al. (27) evaluated Doppler parameters of the umbilical artery (UmA), uterine artery (UA), and fetal middle cerebral artery (MCA) before and after MgSO4 administration in women with severe preeclampsia. They showed that there was a significant difference between maternal systolic blood pressure, diastolic blood pressure, and heart rate before and after administration of MgSO4 in the studied patients. There was a significant difference between umbilical artery, middle cerebral artery, and uterine artery Doppler parameters before and after administration of MgSO4 in the studied patients. There was no significant difference between umbilical artery/middle cerebral artery with regard to RI and PI. However, there was significant difference with regard to the S/D ratio. Finally, the decrease in the values of Doppler parameters before and after administration of MgSO4 was more in the middle cerebral artery than in the umbilical artery (27). Souza et al. (28) evaluated Doppler velocimetry parameters (resistance index [RI], pulsatility index [PI] and systolic/diastolic [S/D] ratio) of maternal-fetal circulation before and after magnesium sulfate administration in pregnant women with severe preeclampsia. They showed that there was a statistically significant increase in mean maternal heart rate and a statistically significant decrease in systolic, diastolic and mean maternal blood pressure before and after administration of magnesium sulfate ($p < 0.001$). Resistance index decreased in the umbilical ($p = 0.003$; 95% CI: 0.008 - 0.03) and middle cerebral artery ($p = 0.001$; 95% CI: 0.01 - 0.05) and in both uterine arteries and there was a significant reduction in the PI and S/D ratio in all the arteries assessed (28). Rantonen et al. (29) showed that after the use of MgSO4 for the prevention of convulsions, only women with severe preeclampsia have shown a reduction of systolic and diastolic blood pressure, while the maternal heart rate was increased ($p < 0.001$). They also found a significant decrease after the use of MgSO4 for PI of the uterine artery ($p < 0.001$), umbilical artery ($p = 0.008$) and fetal middle cerebral arteries ($p < 0.001$), with a reduction of 0.1, 0.05, and 0.15, respectively. Therefore, the hypothesis that the vasodilator effect of the magnesium was more evident where the higher vascular resistance was found was confirmed (29).

CONCLUSION

We can conclude that MgSO4 proved to cause many hemodynamic changes as it has vasodilator effect on maternal and fetal blood vessels. Doppler indices in the umbilical, uterine (decrease in PI and RI) and the middle cerebral (increase in PI and RI) arteries are significantly changed after administration of MgSO4 in pregnant women with different etiologies before 32 weeks and it can be given to these group patients. Because of lower sample size in our study, it is recommended to perform this study in large-scale population and older gestational age between 32 to 37 gestational weeks.

REFERENCES

1. Euser AG, Cipolla MJ. Magnesium sulfate for the treatment of eclampsia: a brief review. Stroke. 2009;40(4):1169-75.
2. Witlin A, Sibai B. Magnesium sulfate therapy in preeclampsia and eclampsia. Obstetrics & Gynecology. 1998;92(5):883-9.
3. Euser AG, Bullinger L, Cipolla MJ. Magnesium sulphate treatment decreases blood–brain barrier permeability during acute hypertension in pregnant rats. Experimental physiology. 2008;93(2):254-61.
4. Altman D, Carroli G, Duley L, Farrell B, Moodley J, Neilson J, et al. Do women with pre-eclampsia, and their babies, benefit from magnesium sulphate? The Magpie Trial: a randomised placebo-controlled trial. Lancet (London, England). 2002;359(9321):1877-90.
5. Reynolds JD, Chestnut DH, Dexter F, McGrath J, Penning DH. Magnesium sulfate adversely affects fetal lamb survival and blocks fetal cerebral blood flow response during maternal hemorrhage. Anesthesia & Analgesia. 1996;83(3):493-9.
6. Hauth J, Goldenberg R, Nelson K, DuBard M, Peralta M, Gaudier F. Reduction of cerebral palsy with maternal MgSO4 treatment in newborns weighing 500-1000 g. Am J Obstet Gynecol. 1995;172(1 Pt 2):419.
7. Nelson KB, Grether JK. Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants? Pediatrics. 1995;95(2):263-9.
8. Sibai BM. Magnesium sulfate prophylaxis in preeclampsia: Lessons learned from recent trials. American Journal of Obstetrics & Gynecology. 2004;190(6):1520-6.
9. Livingston JC, Livingston LW, Ramsey R, Mabie BC, Sibai BM. Magnesium sulfate in women with mild preeclampsia: a randomized controlled trial. Obstetrics & Gynecology. 2003;101(2):217-20.
10. Dasgupta S, Ghosh D, Seal SL, Kamilya G, Karmakar M, Saha D. Randomized controlled study comparing effect of magnesium sulfate with placebo on fetal umbilical artery and middle cerebral artery blood flow in mild preeclampsia at >/= 34 weeks gestational age. The journal of obstetrics and gynaecology research. 2012;38(5):763-71.
11. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. Obstetrics and gynecology. 1992;79(3):416-20.

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12. Ebrashy A, Azmy O, Ibrahim M, Waly M, Edris A. Middle cerebral/umbilical artery resistance index ratio as sensitive parameter for fetal well-being and neonatal outcome in patients with preeclampsia: case-control study. Croatian medical journal. 2005;46(5).
13. Bahlmann F, Reinhard I, Krummenauer F, Neubert S, Macchiella D, Wellek S. Blood flow velocity waveforms of the fetal middle cerebral artery in a normal population: reference values from 18 weeks to 42 weeks of gestation. Journal of perinatal medicine. 2002;30(6):490-501.
14. Tarzamni M-K, Nezami N, Gatreh-Samani F, Vahedinia S, Tarzamni M. Doppler waveform indices of fetal middle cerebral artery in normal 20 to 40 weeks pregnancies. Arch Iran Med. 2009;12(1):29-34.
15. Karsdorp V, Van Vugt J, Van Geijn H, Kostense P, Arduim D, Montenegro N, et al. Clinical significance of absent or reversed end diastolic velocity waveforms in umbilical artery. The Lancet. 1994;344(8938):1664-8.
16. Mihu D, Diculescu D, Costin N, Mihu CM, Blaga L, Ciortea R, et al. Applications of Doppler ultrasound during labor. Medical ultrasonography. 2011;13(2):141.
17. Rana MJ, Amanullah A, Farooq O. The role of umbilical artery Doppler in detection and management of fetal growth retardation. Biomedica. 2005; 21:4-7.
18. Divon MY. Umbilical artery Doppler velocimetry: clinical utility in high-risk pregnancies. American Journal of Obstetrics & Gynecology. 1996;174(1):10-4.
19. Vyas S, Nicolaides K, Bower S, Campbell S. Middle cerebral artery flow velocity waveforms in fetal hypoxaemia. BJOG: An International Journal of Obstetrics &Gynaecology. 1990;97(9):797-803.
20. Keeley MM, Wade RV, Laurent SL, Hamann VD. Alterations in maternal-fetal Doppler flow velocity waveforms in preterm labor patients undergoing magnesium sulfate tocolysis. Obstetrics and gynecology. 1993;81(2):191-4.
21. Cunningham FG, Lindheimer MD. Hypertension in pregnancy. New England Journal of Medicine. 1992;326(14):927-32.
22. Dubiel M, Gudmundsson S, Gunnarsson G, Marsál K. Middle cerebral artery velocimetry as a predictor of hypoxemia in fetuses with increased resistance to blood flow in the umbilical artery. Early human development. 1997;47(2):177-84.
23. Skajaa K, Svane D, Andersson K-E, Forman A. Effects of magnesium and isradipine on contractile activation induced by the thromboxane A2 analog U46619 in human uteroplacental arteries in term pregnancy. American Journal of Obstetrics & Gynecology. 1990;163(4):1323-33.
24. Lacin S, Demir N, Koyuncu F, Goktay Y. Value of intraplacental villous artery Doppler measurements in severe preeclampsia. Journal of postgraduate medicine. 1996;42(4):101.
25. Belfort MA, Saade GR, Kenneth JM. The effect of magnesium sulfate on maternal and fetal blood flow in pregnancy-induced hypertension. ActaObstetricia et GynecologicaScandinavica. 1993;72(7):526-30.
26. Twickler DM, McIntire DD, Alexander JM, Leveno KJ. Effects of magnesium sulfate on preterm fetal cerebral blood flow using Doppler analysis: a randomized controlled trial. Obstet Gynecol. 2010;115(1):21-5.
27. Maged AM, Hashem AM, Gad Allah SH, Mahy ME, Mostafa WA, Kotb A. The effect of loading dose of magnesium sulfate on uterine, umbilical, and fetal middle cerebral arteries Doppler in women with severe preeclampsia: A case control study. Hypertension in pregnancy. 2016;35(1):91-9.
28. Souza AS, Amorim MM, Coutinho IC, Lima MM, Noronha Neto C, Figueroa JN. Effect of the loading dose of magnesium sulfate (MgSO4) on the parameters of Doppler flow velocity in the uterine, umbilical and middle cerebral arteries in severe preeclampsia. Hypertension in pregnancy. 2010;29(2):123-34.
29. Rantonen T, Kääpä P, Grönlund J, Ekblad U, Helenius H, Kero P, et al. Maternal magnesium sulfate treatment is associated with reduced brain-blood flow perfusion in preterm infants. Critical care medicine. 2001;29(7):1460-5.

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