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The Effect of Magnesium Sulfate on Bleeding Time and Nitric Oxide Production in Preeclamsia

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Abstract: Preeclampsia is a disease regarding with altered vascular reactivity leading to hypertension of the mother and metabolic alterations in the fetus. This study aimed to assess nitric oxide and bleeding time following administration of magnesium sulfate to preeclamtic patients compared to normotensive pregnant women. A total of 112 subjects (56 preeclamtic patients and 56 normotensive pregnant controls) were enrolled in this case-control study. Cases and controls were matched for age, BMI, gestational age, parity and gravidity. Total concentration of nitrite and nitrate (NOx) was measured before and during magnesium sulfate ($MgSO_4$) treatment using a modified Griess-based method. Results: Systolic and diastolic blood pressures were significantly decreased during $MgSO_4$ treatment in preeclamtic patients ($p<0.0001$). NOx levels were significantly increased in preeclamtic women after $MgSO_4$ administration (33.7±18.5 vs. 50.2±21.6, $p<0.0001$) but it was not seen in normotensive parturients (52.4±28.9 vs. 57.3±21.7, $p = 0.362$). The bleeding time was scarcely increased following magnesium sulfate treatment in preeclamptic patients compared to normotensive pregnant women but it was not significant ($p = 0.18$). In addition, there was only a significantly reverse correlation between NOx levels and systolic or diastolic blood pressure in preeclamtic parturients after $MgSO_4$ treatment ($r = -0384$; $p = 0.003$ and $r = -0.29$; $p = 0.03$, respectively). This study demonstrates that administrating $MgSO_4$ to preeclamtic patients induced significant changes in NOx production which had a major role in modulating vasculature changes in preeclamsia.

Key words: Preeclamsia, pregnancy, nitric oxide, bleeding time, blood pressure

INTRODUCTION

Preeclampsia is a pregnancy-associated multisystem disorder that affects more than 3-5% of all pregnancies (Khalil *et al*., 2009). It remains a leading cause of maternal and naonatal morbidity and mortality in the world (Friedman *et al*., 1991). Reduced placental perfusion in the early stages of pregnancy is a main event in the development of this condition. Preeclamsia is associated with a complex of coagulation abnormalities which is due to an increased in platelet function, fibrinolytic system activation (Dusse *et al*., 2010) thrombin formation (Belfort *et al*., 2006) and accelerated a hypercoagulable state. Meanwhile, many preeclamptic women have at least some evidence of an abnormal clotting cascade (Benedetto *et al*., 1989).

Nitric Oxide (NO) which produce from placenta endothelial cells and platelets, has a major role in physiological vascular adaptation occurs during normal pregnancy such as an increasing in hemodynomic parameters in presence of decreased vascular resistancy (Many *et al*., 2000; Vatish *et al*., 2006). NO induces vasodilatation of placenta (Bachetti *et al*., 2004) inhibits platelet aggregation (Jang *et al*., 2002) is involved in angiogenesis, acts as a neurotransmitter and prevents the adhesion of platelets to endothelial cells (Gladwin, 2005; Lowe, 2000). Altered synthesis and/or biological actions of NO have been related with abnormal blood flow in preeclampsia and gestational diabetes (Casanello *et al*., 2007). Abnormally elevated levels of NO converted metabolites have been reported in various biological fluids from preeclamptic pregnancies

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(Von Mandach *et al*., 2003). Deficient NO formation has been implicated in hypertensive disorders of pregnancy.

Magnesium sulfate ($MgSO_4$), which is used as a therapeutic modality for the prevention of seizures in pregnant women with preeclampsia, has anticoagulant and antiplatelet effects (Briel *et al*., 1987). In addition to its anticonvulsive effects, $MgSO_4$ decreases blood pressure through a still unknown mechanism (Touyz, 2003; Souza *et al*., 2010). $MgSO_4$ has shown the ability to inhibit arterial thrombus formation in some experimental animal studies and treatment with magnesium might lower the risk of thromboembolic-related disorders (Sheu *et al*., 2003). The results of studying the effect of $MgSO_4$ infusion on bleeding time in preeclamsia have been controversial. Kynczl-Leisure showed an increased bleeding time in the preeclamtic women received $MgSO_4$ (Kynczl-Leisure and Cibils, 1996) however other studies have reported a decreased in bleeding time (Ravn *et al*., 1996) or without significant affect (Falck *et al*., 1999; Rukshin *et al*., 2001). However, there is no published study has determined and correlated bleeding time, blood pressure and NO levels in a single sample of preeclamptic patient through $MgSO_4$ administration. Therefore, the present study was designed to measure all these variables in normotensive pregnant and preeclamptic women.

MATERIALS AND METHODS

Study population: A total of 112 pregnant women including 56 preeclamtic patients and 56 normotensive pregnant women, referred for their prenatal care at the Department of Obstetrics and Gynecology, Imam Khomeini Hospital, Sari, North of Iran during Nov. 2008 to May 2009, were recruited in this case control study. All subjects were on 20-40 weeks of gestation according to a reliable last normal menstrual period date or sonographic reports. Control patients were matched with those with preeclampsia for maternal age and gestational age at the time of blood sampling. Preeclampsia was diagnosed as a new onset of hypertension (systolic blood pressure (BP) ≥140 mm Hg or diastolic BP≥90 mm Hg) and proteinuria (>300 mg dL^{-1}) on at least two occasions 6-24 h apart (ACOG Committee on Practice Bulletins-Obstetrics, 2002). Women in labor with ruptured membranes, multiple pregnancy, medical complications including autoimmune disorders, anti-platelet antibodies, diabetes mellitus, thrombocytopenia, inflammatory conditions and those having a history of coagulopathy and cases with bleeding time more than 420 seconds were excluded from study. All subjects gave written or oral informed consent before participating and the protocol was approved by the Ethics Research Committee of Mazandaran University of Medical Sciences.

Baseline laboratory data included an initial bleeding time, platelet count and serum creatinin. All preeclamtic patients received a 4 g bolus of IV $MgSO_4$ diluted in 100 mL of 5% dextrose solution over 20 min, followed by an infusion of 2 g h^{-1} for 24 h. Bleeding time was measured by means of a modified Ivy method (Pagana and Pagana, 2002) with a Surgicatt device (International Technidyne, Edison, N.J.) before $MgSO_4$ was given and also 24 h after it infused. To minimize technical variation, all bleeding time measurements were blindly done by one investigator. The bleeding time was recorded as mean occasions of every 30 sec until bleeding stopped. Upon hospital admission, in two occasions, 10 mL blood samples obtained from each parturient. Blood samples were collected in citrated vials before commencement of the magnesium bolus and 40 h after the administration of the $MgSO_4$. Blood sampling was done in control group as the same times as preeclamtic group. Serum aliquots were immediately frozen at -80°C until assayed. Blood pressure was also recorded just before sampling.

Measurement of Total Plasma Nitrite Level (NOx): Since NO is unstable and rapidly converted to nitrates and nitrites, it is necessary to determine both total concentrations nitrite and nitrate in samples. In the present study, all nitrate was converted to nitrite using chemical reduction by vanadium chloride. Total concentration of nitrite and nitrate (NOx) was determined in thawed serum supernatant by a modified Griess reaction. Briefly, serum samples were diluted four fold with distilled water and deproteinized by adding 1/20th volume of zinc sulfate (300 g L^{-1}) to give a final concentration of 15 g L^{-1}. After centrifugation at 10000 g for 5 mm at room temperature, 100 μL of supernatant was applied to a microtiter plate well, followed by 100 μL vanadium chloride (400 mg were prepared in 50 mL 1M HCL) and 50 μL of Griess reagent (1 g L^{-1} sulfanilamide, 25 g L^{-1} phosphoric acid and 0.1 g L^{-1} N-1-naphthylethylenediamine). After 10 min of color development at room temperature, the absorbance was measured on a microplate reader at a wavelength of 540 nm. Each sample was assayed in duplicate wells. Calibration curves were made with sodium nitrite in distilled water (linear range 0-100 μmol L^{-1}). The detection limit of the assay is 1.5 μmol.

Statistical analysis: Data are expressed as Mean±SD for variables with normal distribution. Differences between groups were analyzed using the independent t-test or

χ^2-test, appropriately. In addition, differences into one group were analyzed using the paired-sample t-test. Pearson's correlation test was used to study associations between NO levels and other variables. Differences with P-values below 0.05 were considered significant.

RESULTS

Table 1 summarizes the clinical characteristics of the 112 subjects enrolled in the present study. There were no significant differences in age, gestational age at sampling, Body Mass Index (BMI), parity and gravidity between preeclamtic patients and normotensive pregnant women ($p<0.05$). Majority of study groups were primigravidia. However, women with preeclamsia had higher creatin in levels and lower platelet count than those in the control group ($p<0.05$). As expected, higher systolic and diastolic blood pressures were found in women with preeclampsia compared with the control group ($p<0.0001$).

Table 1: Characteristics of pregnant women with preeclampsia and healthy control

	Preeclamsia	Control	P-value
Age (Mean±SD)	27.20±6	27.10±5.3	0.947
BMI	29.35±3.01	29.43±2.8	0.89
Platelet (mm^3)	198.34±56.84	223.30±67.67	0.047
Creatinin (mg dL^{-1})	0.84±0.1	0.66±0.122	<0.0001
Systolic blood pressure (mm Hg)	164.46±18.31	110.10±9.27	<0.0001
Diastolic blood pressure (mm Hg)	99.73±6.7.41	67.41±8.74	<0.0001
Gravidity			
1	48 (85.7)	45 (80.4)	
2	4 (7.1)	7 (12.5)	0.63
3	4 (7.2)	4 (7.2)	
Parity			
0	51 (91.1)	46 (82.1)	
1	1 (1.8)	6 (10.7)	0.147
2	4 (7.1)	4 (7.1)	

Values are the Mean±SEM or no. (%). P values were determined by application of Student's t-test for continuous variables and by χ^2 test for categorical variables

The effect of $MgSO_4$ on the bleeding time and nitric oxide levels are shown in (Table 2). Administration of Magnesium sulfate to preeclamtic group leads to significant decreased in bleeding time when compared to healthy pregnant women (<0.0001). However, comparison of bleeding time before and after $MgSO_4$ treatment revealed an increased in coagulation time in preeclamtic patients (Table 3), however, it was not significantly different ($p = 0.18$). In addition, the based line of serum levels of nitric oxide was significantly lower in preeclamtic group than those of control group (<0.0001). Nonetheless, after magnesium treatment, there was no significant difference in NOx production in preeclamtic patients compared to normotensive controls ($p = 0.177$). After $MgSO_4$ treatment, the serum levels of NOx was significantly increased in preeclamtic patients (33.75±18.48 vs. 50.78±22.3 μmol mL^{-1}, $p<0.0001$). However, this high NOx production did not meet statistical significanct difference, in preeclamtic group compared to controls ($p = 0.177$). On the other hand, NOx levels were significantly different only in peeclamtic women before and after magnesium infusion ($p<0.0001$ and $p = 0.362$, respectively).

To address the possibility that any relationships exist between the circulating concentrations of NOx and bleeding time and systolic or diastolic blood pressure, we carried out correlation analysis between these variables. As it was shown in parts A and B of Fig. 1, there is no correlation between NOx levels and systolic or diastolic blood pressure before magnesium sulfate administration in preeclamtic patients ($p = 0.18$).

Table 2: Effect of magnesium on bleeding time and nitric oxide production in preeclamtic patients compared to healthy pregnant

	Before			After		
	Preeclamsia	Control	p-value	Preeclamsia	Control	p-value
Bleeding time (sec)	268.2±67.6	284.50±60.9	0.182	274.60±56.1	326.96±60.03	<0.0001
Systolic BP (mm Hg)	164.5±18.3	110.10±9.3	<0.0001	131.70±11.8	109.60±10.6	<0.0001
Diastolic BP (mm Hg)	99.70±11.2	67.40±11.2	<0.0001	85.50±8.1	70.50±15.9	<0.0001
Nitric oxide (μmol mL^{-1})	33.75±18.48	52.45±26.86	<0.0001	50.78±22.3	57.71±31.01	0.177

p values were determined by Student T test; P value indicates a significant difference ($p<0.05$) between both groups

Table 3: Evaluation of laboratory findings in patients with preeclamsia and healthy controls following magnesium infusion

	Preeclamsia			Control		
	Before	After	p-value	Before	After	p-value
Bleeding time (sec)	268.20±67.6	274.60±56.1	0.18	284.50±60.9	326.96±60.0	<0.0001
Systolic BP (mm Hg)	164.50±18.3	131.70±11.8	<0.0001	110.10±9.3	109.60±10.6	0.81
Diastolic BP (mm Hg)	99.70±11.2	85.50±8.1	0.0001	67.40±11.2	70.50±15.9	0.154
Nitric oxide (μmol mL^{-1})	33.75±18.48	50.78±22.3	<0.0001	52.45±26.86	57.71±31.01	0.362

P values were determined by Student T test; P value indicates a significant difference ($p<0.05$) between both groups

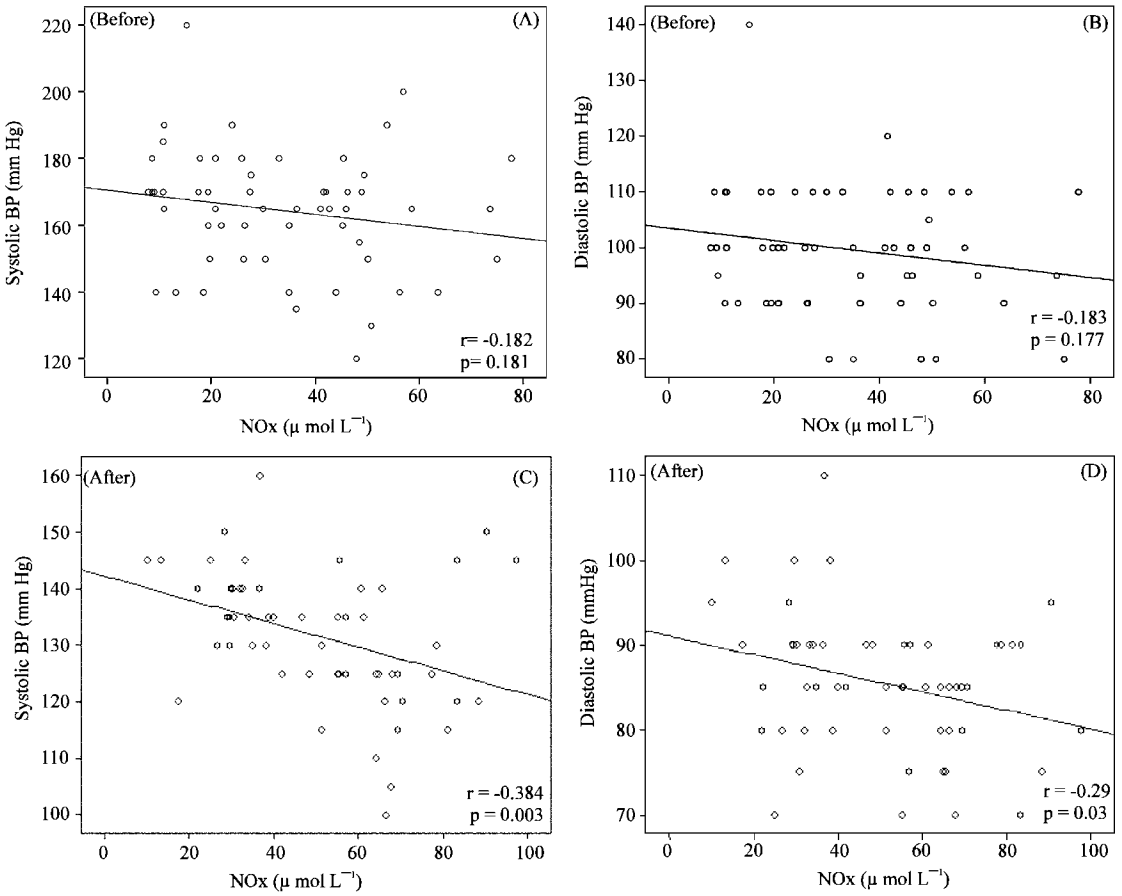

Fig. 1: Correlation between NOx and systolic or diastolic blood pressure in preeclamtic patients before (A and B parts) and after (C and D parts) magnesium treatment. There is only a reverse significant correlation between NOx concentration and systolic or diastolic blood pressure after $MgSO_4$ treatment

Nonetheless, after $MgSO_4$ treatment, NOx levels had a significantly reverse correlation to systolic or diastolic BP; $p = 0.003$ and 0.03, respectively (parts C and D of Fig. 1). We could not find any correlation between NOx concentration and bleeding time, before and after magnesium treatment.

DISCUSSION

A main finding of this study is that lower plasma NOx levels in parturients with preeclampsia compared with normotensive healthy pregnant women that can be compensated by magnesium administration. In addition, there is only a significantly reverse correlation between NOx levels and blood pressure in preeclamtic patients received magnesium sulfate treatment. Since nitric oxide converts rapidly to its metabolites (nitrite and nitrate) and Griess reaction could only measure nitrite metabolite, in this study we first converted nitrate to nitrite to have more perception about nitric oxide concentration.

Through normal pregnancy, there is several hemodynamic adaptations include increased cardiac output without increasing in materno-fetal vascular resistancy. It is postulated that nitric oxide, by dilating of systemic vasculature, has a major role in the lack of increase in arterial blood pressure in normal pregnant women (Sladek *et al*., 1997; Sandrim *et al*., 2008). Systolic and diastolic blood pressures of preeclamtic parturients were significantly after magnesium sulfate administration. These results are similar to those reported by other investigators, suggesting that acute administration of magnesium sulfate in women with preeclampsia decreases systemic vascular resistance and blood pressure and increases cardiac output (Cotton *et al*., 1984). Increased No production following magnesium sulfate infusion in preeclamtic women might be one of the mechanisms in regulating blood pressure. However, we could not find a significant difference in serum levels of NO in preeclamsia compared to normotensive pregnant women. It was suggested that significant decreased in blood pressure

after infusing magnesium sulfate is a synergic mechanism of NO production and other regulatory mechanisms. This conclusion was according to the results of other study demonstrated magnesium sulphate acts as a vasodilator by increasing the synthesis of prostacyclin, as well as inhibiting angiotensin converting enzyme activity (Elsharnouby and Elsharnouby, 2006).

Endothelial dysfunction and injury most likely result in hypertension, proteinuria and other systemic manifestations of the preeclamsia. These modifications lead to ischemic and hypoperfused placentas (Steinberg *et al*., 2009). It is also reported that dysfunction of endothelial cells can contribute to inappropriate vasoconstriction and platelet aggregation, which are early signs of hypertension and thrombosis (Vane and Botting, 1992).

It is demonstrated that blocking of NO production in animal models causes microvasculature changes resemble to preeclamsia (Pandhi and Malhotra, 2002). The decreased NOx levels in preeclamtic parturients compare to normotensive pregnant women in our study is also reinforced involvement of nitric oxide in normal pregnancy.

NO levels are maintained by its production by Nitric Oxide Synthase (NOS), but several factors could control NO plasma levels, including free radicals and NOS substrate concentrations. It was demonstrated that decreasing levels of endothelial NOS (eNOS) but increasing inducible NOS (iNOS) during preeclamsia may lead to low NO concentration in preeclamtic patients (Ariza *et al*., 2009). Here, we showed an increased in the levels of NOx production in preeclamtic parturients 24 h after $MgSO_4$ administration. As the levels of NOx is correlated by the eNOS activity, it seems $MgSO_4$ has a profoundly effect on eNOS activity. Recently, Ariza *et al*. (2009) showed that magnesium has opposite effects on iNOS and eNOS in mild preeclamsia (Ariza *et al*., 2009). Another postulation that may be made on effect of magnesium on preeclamsia is due to decreasing free radicals, NOS inhibitors. It was reported that magnesium gluconate had an anti-radical and cytoprotective effects *in vitro* (Mak *et al*., 2000). We found a significantly reverse correlation between NOx levels and blood pressure in preeclamtic parturients after receiving $MgSO_4$. On the other hand, $MgSO_4$ treatment significantly reduced systolic and diastolic blood pressure in preeclamtic patients. This finding suggects that the antihypertensive effect of $MgSO_4$ may be mediated, at least in part, by increasing serum NO levels.

Otherwise, we did not find a significantly positive association between NOx levels and bleeding time in preeclamtic patients. This finding was shown that coagulation is extremely complex process involving many interacting factors other than platlets. However, a scarcely increased bleeding time in patients with preeclamsia treated by $MgSO_4$, was showed an indirect effect of high NO production in coagulation.

There is a limitation to this study that should be mentioned. It is possible that our results may be false positives due to the nitrite/nitrate level does not definitely reflect bioactive amount of NO. It has shown nitrite/nitrate plasma levels are affected by the dietary consumption of nitrite/nitrate. Therefore, to introduce a well-defined correlation between NOx concentration and preeclamsia, eNOS activity should be targeted for further investigation.

In conclusion, this study demonstrates that $MgSO_4$ treatment to preeclamtic pregnant women induced significant changes in NOx production which had a major role in modulating vasculature changes in preeclamsia

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