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

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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 preeclamptic patients compared to normotensive pregnant women. A total of 112 subjects (56 preeclamptic 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 (MgSO4) treatment using a modified Griess-based method. Results: Systolic and diastolic blood pressures were significantly decreased during MgSO4 treatment in preeclamptic patients (p<0.0001). NOx levels were significantly increased in preeclamptic women after MgSO4 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 preeclamptic parturients after MgSO4 treatment (r = -0384; p = 0.003 and r = -0.29; p = 0.03, respectively). This study demonstrates that administering MgSO4 to preeclamptic patients induced significant changes in NOx production which had a major role in modulating vasculature changes in preeclampsia.

Key words: Preeclampsia, 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 neonatal 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. Preeclampsia 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 hemodynamic parameters in presence of decreased vascular resistancy (Mary 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 (Casamello et al., 2007). Abnormally elevated levels of NO converted metabolites have been reported in various biological fluids from preeclamptic pregnancies.
(Von Mandach et al., 2003). Deficient NO formation has been implicated in hypertensive disorders of pregnancy.

Magnesium sulfate (MgSO₄), which is used as a therapeutic modality for the prevention of seizures in pregnant women with preeclampsia, has anticoagulant and antithrombotic effects (Briel et al., 1987). In addition to its anticoagulant effects, MgSO₄ decreases blood pressure through a still unknown mechanism (Touyz, 2003; Souza et al., 2010). MgSO₄ 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 MgSO₄ infusion on bleeding time in preeclampsia have been controversial. Kyncl-Leisure showed an increased bleeding time in the preeclamptic women received MgSO₄ (Kyncl-Leisure and Cibils, 1996) however other studies have reported a decreased in bleeding time (Ravn et al., 1996) or without significant affect (Falek 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 MgSO₄ 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 preeclamptic 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 preeclamptic patients received a 4 g bolus of IV MgSO₄ 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 Surgicat device (International Technidyne, Edison, N.J.) before MgSO₄ 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 MgSO₄. 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 min 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-naphthylethenediamine). 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
\( \chi^2 \)-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 preeclampsia and normotensive pregnant women (p<0.05). Majority of study groups were primigravidia. However, women with preeclampsia 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).

The effect of MgSO\textsubscript{4} on the bleeding time and nitric oxide levels are shown in Table 2. Administration of Magnesium sulfate to preeclamptic 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\textsubscript{4} treatment revealed an increased in coagulation time in preeclamptic patients (Table 3), however, it was not significantly different (p=0.18). In addition, the baseline of serum levels of nitric oxide was significantly lower in preeclamptic group than those of control group (<0.0001). Nonetheless, after magnesium treatment, there was no significant difference in NOx production in preeclamptic patients compared to normotensive controls (p=0.177). After MgSO\textsubscript{4} treatment, the serum levels of NOx was significantly increased in preeclamptic patients (33.75±18.48 vs. 50.78±22.3 \( \mu \)mol mL\textsuperscript{-1}, p<0.0001). However, this high NOx production did not meet statistical significant difference, in preeclamptic group compared to controls (p=0.177). On the other hand, NOx levels were significantly different only in preeclamptic 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 preeclamptic patients (p=0.18).

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

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean±SD)</td>
<td>27.2±6</td>
<td>27.1±5.3</td>
<td>0.947</td>
</tr>
<tr>
<td>BMI</td>
<td>29.3±3.01</td>
<td>29.4±2.8</td>
<td>0.89</td>
</tr>
<tr>
<td>Platelet (mm\textsuperscript{3})</td>
<td>108.1±56.84</td>
<td>223.3±67.67</td>
<td>0.047</td>
</tr>
<tr>
<td>Creatin (mg dl\textsuperscript{-1})</td>
<td>0.84±0.1</td>
<td>0.66±0.122</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>164.4±18.31</td>
<td>110.1±9.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>99.7±6.74</td>
<td>67.4±8.74</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gravity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>48 (85.7)</td>
<td>45 (80.4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4 (7.1)</td>
<td>7 (12.5)</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>4 (7.2)</td>
<td>4 (7.2)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>51 (91.1)</td>
<td>46 (82.1)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (1.8)</td>
<td>6 (10.7)</td>
<td>0.147</td>
</tr>
<tr>
<td>2</td>
<td>4 (7.1)</td>
<td>4 (7.1)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time (sec)</td>
<td>268.2±67.6</td>
<td>284.5±60.9</td>
<td>0.182</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>164.5±18.3</td>
<td>110.1±9.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>99.7±11.2</td>
<td>67.4±11.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nitric oxide (( \mu )mol mL\textsuperscript{-1})</td>
<td>33.75±18.48</td>
<td>52.45±26.86</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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 preeclampsia and healthy controls following magnesium infusion

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time (sec)</td>
<td>268.2±67.6</td>
<td>274.6±56.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>164.5±18.3</td>
<td>131.7±11.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>99.7±11.2</td>
<td>67.4±11.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Nitric oxide (( \mu )mol mL\textsuperscript{-1})</td>
<td>33.75±18.48</td>
<td>52.45±26.86</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P values were determined by Student T-test; P value indicates a significant difference (p<0.05) between both groups.
Nonetheless, after MgSO₄ 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 preeclamptic 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 preeclamptic 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 preeclampsia 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 (Elshamouby and Elshamouby, 2006).

Endothelial dysfunction and injury most likely result in hypertension, proteinuria and other systemic manifestations of the preeclampsia. 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 preeclampsia (Pandhi and Mahotra, 2002). The decreased NOX levels in preeclamptic 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 preeclampsia may lead to low NO concentration in preeclamptic patients (Ariza et al., 2009). Here, we showed an increased in the levels of NOX production in preeclamptic parturients 24 h after MgSO4 administration. As the levels of NOX is correlated by the eNOS activity, it seems MgSO4 has a profoundly effect on eNOS activity. Recently, Ariza et al. (2009) showed that magnesium has opposite effects on iNOS and eNOS in mild preeclampsia (Ariza et al., 2009). Another postulation that may be made on effect of magnesium on preeclampsia 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 preeclamptic parturients after receiving MgSO4. On the other hand, MgSO4 treatment significantly reduced systolic and diastolic blood pressure in preeclamptic patients. This finding suggests that the antihypertensive effect of MgSO4 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 preeclamptic patients. This finding was shown that coagulation is extremely complex process involving many interacting factors other than platelets. However, a scarcely increased bleeding time in patients with preeclampsia treated by MgSO4, 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 preeclampsia, eNOS activity should be targeted for further investigation.

In conclusion, this study demonstrates that MgSO4 treatment to preeclamptic pregnant women induced significant changes in NOX production which had a major role in modulating vasculature changes in preeclampsia

REFERENCES


