

Cerebral spinal fluid and serum ionized magnesium and calcium levels in preeclamptic women during administration of magnesium sulfate

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Objective: To study the distribution of ionized and total magnesium (Mg) in serum and cerebral spinal fluid (CSF) in preeclamptic women receiving MgSO₄ and how this treatment affects the ionized calcium (Ca²⁺) and ionized Ca:Mg ratios compared with healthy nonpregnant women and pregnant control women (HP).

Design: Controlled clinical study.

Setting: An academic medical center.

Patient(s): African-American women older than 20 and less than 35 years. The pregnant preeclamptic study and pregnant control groups each consisted of 16 women; the nonpregnant group consisted of 10 subjects.

Intervention(s): The preeclamptic women received a 6-g bolus of MgSO₄ IV started at least 4.5 hours before delivery during 15–20 minutes, then 2 g/h baseline.

Main Outcome Measure(s): The CSF and serum levels of Ca²⁺ and Mg²⁺ and total Mg were measured in all three groups of women. The Ca²⁺:Mg²⁺ ratios were determined. Physiologic monitoring was done and recorded every 4 hours where appropriate. Bloods were drawn every 6 hours for complete blood count, metabolic panel, lactate dehydrogenase, uric acid, and electrolytes. Serum pH, total Mg, Apgar scores, and general health of the infants born to preeclamptic mothers given MgSO₄ were followed.

Result(s): The HP showed a reduction in mean serum ionized and total Mg, increase in ionized Ca, and a large increase in Ca²⁺:Mg²⁺ ratios compared with healthy nonpregnant women. Although the CSF ionized and total Mg and Ca²⁺:Mg²⁺ ratios were not altered with MgSO₄ treatment in the preeclamptic women receiving MgSO₄, the mean serum Mg values increased 3-fold. All infants were full-term, regardless of MgSO₄ treatment, and normal with respect to birth weight, Apgar scores, blood pH, total Mg, and neurologic scores.

Conclusion(s): The data indicate that there is a direct relationship between the serum and CSF Ca²⁺:Mg²⁺ ratios in HP and this ratio may be crucial in preventing vascular and neurologic complications in preeclampsia–eclampsia. (Fertil Steril® 2010;94:276–82. ©2010 by American Society for Reproductive Medicine.)

Key Words: Cerebral spinal fluid, preeclampsia, MgSO₄ treatment, ionized magnesium, ionized calcium, ionized calcium:magnesium ratio, infants born to preeclamptic mothers

Eclampsia–preeclampsia is a potentially dangerous condition in pregnant women that can result in premature labor, premature birth, growth retardation, convulsions in both mother and fetus, cerebral palsy in the newborn, and sometimes death of mother and fetus (1, 2). The syndrome consists of high blood pressure, edema, increased vascular reactivity to pressors, uteroplacental changes (ischemia, infarctions), cerebral and

visual disturbances, and coagulation defects. Magnesium sulfate (MgSO₄), given IV, has been used successfully for more than 80 years, to minimize the increased vascular reactivity, hypertension, cerebral ischemia, premature labor, and convulsions (1–4).

Hypomagnesemia has been seen in preeclamptic women (5–7). Even normal pregnant women show progressive hypomagnesemia, particularly during the last trimester (8, 9). According to recent dietary surveys, the dietary intake among the population has been steadily declining since 1900, to the point that the magnesium (Mg) balance often is negative (10–13).

There are reports that placentas from women with preeclampsia or eclampsia exhibit decreased Mg content and increased calcium (Ca) content (14, 15). A higher than normal ratio of Ca²⁺:Mg²⁺ has been shown to provoke vasospasm,

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increased vascular reactivity, and decreased blood flow in coronary, cerebral and umbilical-placental blood vessels (16–19). Excess Mg has been demonstrated repeatedly to increase viability of neurons in experimental forms of cerebral ischemia and traumatic brain injury (20–24). This has strengthened the concept that MgSO₄ can act as a neuroprotective agent in strokes and subarachnoid hemorrhage (25–31). After a number of recent studies with MgSO₄ in preeclampsia, there is now international consensus that this agent is the treatment of choice for preeclampsia and eclampsia (32–34). The recent Magpie trial with 10,000 women indicated that MgSO₄ infusions decreased the risk for eclampsia by more than 50% and reduced maternal mortality by half (32). Despite these clinical and experimental achievements, there are no studies to elucidate how ionized and total Mg is distributed in the cerebral spinal fluid (CSF) of preeclamptic women given MgSO₄ nor what effect this compound has on the CSF-ionized Mg and Ca levels.

There are compelling physiological reasons to suspect that biologically active serum and CSF Mg, as well as Ca, may modulate seizure activity in preeclamptic and eclamptic subjects. Magnesium has an antagonistic effect on the *N*-methyl-D-aspartate (NMDA) receptor (35, 36), which is thought to play a role in many forms of convulsions and epilepsy (37, 38). The activation of the NMDA receptor by excitatory amino acids results in Ca influx (39, 40), long-recognized for its proepileptogenic effects (41, 42). Several studies suggest that low levels of Mg²⁺ or an altered balance between Ca²⁺ and Mg²⁺ may have a precipitating role in seizures, whereas elevated Mg²⁺ levels may inhibit these adverse effects (43). Recently, two of us reported, in a prospective study, that randomly selected seizure patients had a significantly lower mean serum Mg²⁺ but not total Mg and a significantly higher Ca²⁺:Mg²⁺ ratio than control subjects (44).

In the present study, we used ion-selective electrodes to measure levels of the physiologically active Mg and Ca in serum and CSF of preeclamptic women and age-matched nonpregnant and normal pregnant women to: [1] correlate Mg levels of serum with those of CSF in preeclamptic women receiving IV MgSO₄ in therapeutic doses; [2] determine whether the Mg²⁺ crosses the blood–brain barrier after IV MgSO₄; [3] document levels of Mg²⁺ and Ca²⁺ in serum and CSF; and [4] determine whether the serum and CSF Ca²⁺:Mg²⁺ ratios are correlated with IV MgSO₄.

MATERIALS AND METHODS

Patient Population

The study was approved by the Institutional Review Board (IRB) of the University Hospital of Brooklyn and SUNY Downstate Medical Center, and written informed consent was obtained from all patients. All subjects were African-Americans older than 20 and less than 35 years. The pregnant control group consisted of 16 healthy women with uncomplicated pregnancies, whereas the nonpregnant group consisted of 10 subjects, who were admitted to the emergency room

with severe headaches and tested for CSF abnormalities. Only those with no abnormalities were included in this study. The study group consisted of 16 gravid women with preeclampsia diagnosed by classic criteria: hypertension, edema, and proteinuria. Criteria for exclusion of the study were a history of: [1] neurologic disease, [2] renal disease, [3] hypertension, [4] vascular disease, and [5] preterm labor.

Physiological Monitoring

Deep tendon reflexes, respiratory rate, oxygen (O₂) saturation, urine output, heart rate, mean arterial blood pressure, as well as neurologic parameters were monitored and recorded every 4 hours. Bloods were drawn every 6 hours for complete blood count, metabolic panel, lactate dehydrogenase, uric acid, and electrolytes, including Ca and Mg.

Protocol

In all pregnant patients, spinal anesthesia was induced, and a 1-mL sample of clear CSF was collected using a 25-gauge spinal needle. At the same time, a 3-mL sample of blood was obtained by venipuncture from the opposite location of the IV injection site. The CSF and blood samples were drawn into additive-free test tubes under aseptic and as close as possible to anaerobic conditions.

Because there has been some controversy as to the conditions of the infants born to preeclamptic mothers given MgSO₄ (e.g., serum Mg levels, birth weight, neurologic deficits, pH, Apgar scores) (1, 2, 5–7, 45, 46), we followed the babies born to mothers given MgSO₄. Serum pH, total magnesium (TMg), Apgar scores, and general health were carefully monitored.

For the study group, a 6-g bolus of MgSO₄ was administered IV. The Mg treatment was started at least 4.5 hours before delivery (range 4.5–48 hours) during 15–20 minutes, then 2 g/h baseline. Total Mg levels were measured by standard techniques (Kodak DT 60; Ektachem Colorimetric Instruments, Rochester, NY) (47, 48). This method compares favorably with atomic absorption spectrometry. Blood for serum Mg²⁺ and Ca²⁺ was drawn anaerobically into red-stoppered vacutainer tubes, allowed to clot, spun down, and the serum was anaerobically placed into another capped vacutainer tube and stored at -4°C for 24–48 hours in a freezer. Some samples were analyzed within 2 hours after venipuncture. An Mg²⁺ ion-selective electrode with a neutral carrier-based membrane and a Ca²⁺-specific electrode (Nova 8 analyzer; Nova Biochemical, Waltham, MA) were used to measure these ions. The electrodes were used in accordance with established procedures, having an accuracy and precision of approximately 3% (47, 48). Five standards were run before each data collection.

Data Analysis

Data are reported as means ± SEM. One-way analysis of variance (ANOVA) or a Student's *t*-test were used to analyze variables for statistically significant differences between groups. Statistical significance was defined as a *P* value of less than .05.

RESULTS

A total of 32 pregnant patients (healthy and preeclamptic) were enrolled. Each group consisted of 16 subjects. The healthy control pregnant women were 20–34 years of age (26 ± 4.9 years); the preeclamptic women treated with $MgSO_4$ ranged from 20–34 years (25 ± 5 years). The nonpregnant group ranged from 20–42 years (31 ± 6 years). A comparison of the three groups of sera is shown in Table 1. It is clear from the data that the healthy, pregnant group shows significant deficits in mean serum ionized and total Mg when compared to nonpregnant controls. On average, there is a 20% deficit in serum ionized Mg at term compared with nonpregnant controls. The total serum Mg shows almost a 30% deficit. Treatment of the preeclamptic women with IV $MgSO_4$ resulted in an approximate 3-fold increase in serum ionized Mg (range 0.82–1.52 mM/L) and a 3.5-fold increase in total Mg level (range 1.40–3.29 mM/L) when compared with the healthy pregnant women.

With respect to serum ionized Ca, the healthy pregnant women had significantly higher levels than the nonpregnant controls (Table 1), whereas the serum ionized Ca in the $MgSO_4$ -treated preeclamptic women had significantly lower levels, by approximately 10% compared with normal pregnant women and were similar to nonpregnant controls. The healthy, pregnant controls showed a 38% increase in the $Ca^{2+}:Mg^{2+}$ ratios when compared with nonpregnant controls, whereas the preeclamptic-treated group demonstrated a 46% decrease in the $Ca^{2+}:Mg^{2+}$ ratio when compared with nonpregnant women and a 60% decrease in this ratio when compared with healthy pregnant women (Table 1).

In Table 2, the values for mean CSF Mg^{2+} , CSF total Mg, CSF Ca^{2+} , and CSF $Ca^{2+}:Mg^{2+}$ ratios are shown. It is clear from the data that the ionized fraction of Mg in the CSF of normal, nonpregnant women is higher than that observed in healthy pregnant or preeclamptic women. Almost 100% of the Mg is ionized in CSF of the nonpregnant subjects, but this fraction is reduced to about 60% in healthy pregnant and in preeclamptics treated with Mg. However, administration of $MgSO_4$, unlike what is seen for serum, failed to alter

either the ionized Mg, the total Mg, or the ionized fraction in the CSF when comparing the healthy pregnant women with the preeclamptic-treated women.

With respect to CSF Ca^{2+} , nonpregnant women had approximately 15% more of this Ca fraction than did either the normal pregnant subjects or the preeclamptics treated with Mg (Table 2). Surprisingly, and unlike what was found in the serum, the CSF $Ca^{2+}:Mg^{2+}$ ratios are very similar in the nonpregnant, healthy pregnant and preeclamptic women treated with Mg (Fig. 1).

With respect to the infants (all full-term), born to the preeclamptic mothers given $MgSO_4$, we found Apgar scores (8–10), blood pH, total Mg levels, and neurologic scores all to be in the normal ranges and no significant differences in birth weights from the full-term infants born to the normal, healthy mothers ($P > .05$).

DISCUSSION

The possibility that hypermagnesemia may alter the blood–brain barrier and may elevate the CSF levels of Mg, and penetrate the brain parenchyma tissue, is suggested by experimental and human studies (21, 36, 49–52). These studies showed that the neuroprotective attributes of Mg was weakly correlated to elevated serum levels of total Mg. In animal models of cerebral ischemia and hypoxia, as well as traumatic brain injury, treatment with $MgSO_4$ has been shown to reduce infarct size, inhibit neuronal cell death, promote cerebral vasodilation, and attenuate motor deficits (20, 21, 23, 25, 27, 36, 49, 50). Recently, we have shown in experimental animal studies, that bioavailable administration of Mg can attenuate apoptosis by inhibiting DNA fragmentation, inhibiting activation of caspase-3, membrane oxidation, and formation of reactive oxygen species (19). Because, however, the effective sites of Mg^{2+} in the brain are at the neuronal and cerebrovascular levels, it is critical to determine whether induced hypermagnesemia can cause an elevation in brain Mg^{2+} in humans. Measuring total Mg levels does not allow one to determine whether the biologically active Mg is altered significantly (53).

TABLE 1

Ionized magnesium levels and fractions and ionized calcium levels in serum of nonpregnant women, pregnant women, and preeclamptic women after administration of $MgSO_4$.

	Nonpregnant	Healthy pregnant	Preeclamptic + $MgSO_4$
IMg (mM/L)	0.58 ± 0.0003	0.46 ± 0.02^a	1.10 ± 0.05^b
Total Mg (mM/L)	0.84 ± 0.01	0.59 ± 0.04^a	2.05 ± 0.11^b
% IMg	69.0 ± 0.5	78.0 ± 7.82^a	53.6 ± 1.78^b
ICa (mM/L)	1.17 ± 0.006	1.22 ± 0.05^a	1.15 ± 0.02
ICa/Mg	2.02 ± 0.03	2.78 ± 0.17^a	1.09 ± 0.05^b

Note: Values are means \pm SEM. IMg = Mg^{2+} ; ICa = Ca^{2+} ; ICa/Mg = Ca^{2+}/Mg^{2+} .

^aSignificantly different from nonpregnant women ($P < .01$).

^bSignificantly different from nonpregnant and healthy pregnant women ($P < .01$).

Apostol. CSF and serum ionized Mg and Ca in preeclampsia. Fertil Steril 2010.

TABLE 2

Ionized magnesium levels and fractions and ionized calcium levels in cerebral spinal fluid of nonpregnant, healthy pregnant, and preeclamptic women after administration of MgSO₄.

	Nonpregnant	Healthy pregnant	Preeclamptic + MgSO ₄
IMg (mM/L)	1.08 ± 00.3	0.83 ± 0.014 ^a	0.80 ± 0.043 ^a
Total Mg (mM/L)	1.13 ± 0.07	1.31 ± 0.02 ^a	1.34 ± 0.04 ^a
% IMg	95.5 ± 0.05	63.3 ± 2.3 ^a	59.7 ± 2.7 ^a
ICa (mM/L)	1.02 ± 0.03	0.87 ± 0.02 ^a	0.83 ± 0.03 ^a
ICa/Mg	0.94 ± 0.08	1.05 ± 0.02	1.07 ± 0.04

Note: Values are means ± SEM. IMg = Mg²⁺; ICa = Ca²⁺; ICa/Mg = Ca²⁺/Mg²⁺.

^a Significantly different from nonpregnant women ($P < .01$).

Apostol. CSF and serum ionized Mg and Ca in preeclampsia. *Fertil Steril* 2010.

Because it has been shown that preeclampsia–eclampsia may alter the integrity of the blood–brain barrier, administration of a soluble form of Mg and use of ion-specific electrodes should allow one to test this hypothesis. Some previous studies have attempted to gain insight into this question. However, IV MgSO₄ was only administered during short intervals (4–24 hours), not up to 48 hours as in the present study, and the fractions of Mg were not measured. The CSF Mg²⁺ levels have been used in the present study as a marker for brain Mg²⁺ availability. By measuring ionized and total Mg in serum and CSF, we were able to show that a considerable elevation of serum Mg²⁺ above normal for periods up to 48 hours did not alter the CSF Mg²⁺ levels.

Surprisingly, both healthy pregnant and preeclamptic Mg-treated women exhibited reductions in CSF-ionized Ca. Because it is generally believed that low serum Mg levels result in elevated intracellular free Ca in brain neurons, astrocytes, and cerebral vascular smooth muscle cells (15–19, 54–56), one might expect low CSF Mg²⁺ to result in elevated intracellular Ca²⁺, activation of NMDA receptors, and excitation. We show that the elevation of Ca²⁺ in CSF is prevented by hypermagnesemia, an important concept and outcome of our present study.

The Ca²⁺:Mg²⁺ ratios represent an index of tissue calcification potential (16, 18, 19). This was significantly increased in the serum of all normal pregnant subjects compared with nonpregnant controls. These findings support the ideas that: [1] pregnancy itself is a condition of an extracellular decrease of Mg, and [2] this fact may aid in explaining the predisposition to vascular and neuronal abnormalities in pregnancy (17, 57). The fact that infusion of MgSO₄ lowered the serum Ca²⁺:Mg²⁺ ratios 50% and allowed the full-term delivery of normal, healthy babies, would seem to suggest a mechanism for prevention of vascular and neurologic complications.

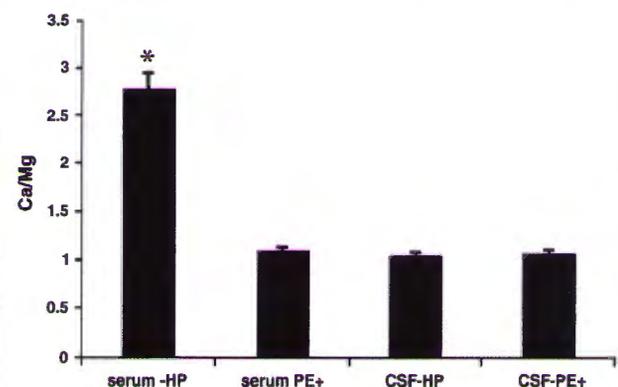
Normally, Mg²⁺ is transported into the central nervous system by an adenosine triphosphate-dependent mechanism. Based on our findings, and those of other investigators on brain trauma patients (51, 52, 58), this transport system appears to be well protected under several stressful conditions,

including mild preeclampsia, keeping homeostasis of brain Mg, and, thus, the concentration of ionized Mg that bathes the neurons intact. In patients with acute brain trauma, in which the blood–brain barrier could have been compromised, a prolonged IV MgSO₄ produced only an 11% increase in ionized magnesium (IMg) (58).

It is apparent from our data that the Ca²⁺:Mg²⁺ ratios in serum are significantly higher in the normal pregnant women than those of either the nonpregnant women or the treated preeclamptics (Fig. 1; $P < .001$). It is also evident (Fig. 2) that there is a direct correlation between the Ca²⁺:Mg²⁺ ratio of serum and CSF for the healthy pregnant women ($P < .05$), whereas there is no such correlation for the treated preeclamptic women (data not shown; $P > .05$). This points to the fact that the concentrations of the two ions in serum act, in a subtle way, to modulate unknown effects leading to neuronal and vascular problems.

FIGURE 1

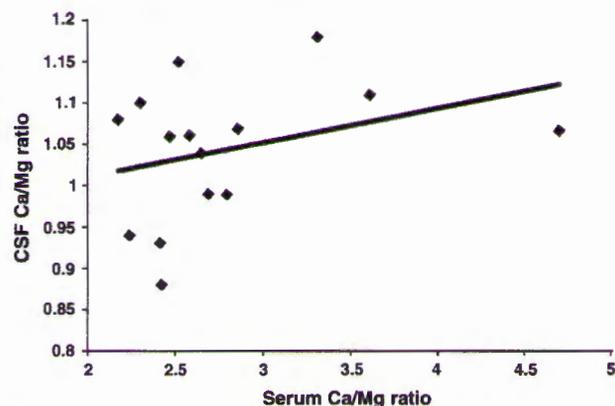
The serum Ca²⁺:Mg²⁺ ratio for the healthy pregnant (HP) group is significantly higher than that of the preeclamptics treated with MgSO₄ (PE+) group and the cerebrospinal fluid (CSF) groups ($P < .001$). Data are means ± SEM.



Apostol. CSF and serum ionized Mg and Ca in preeclampsia. *Fertil Steril* 2010.

FIGURE 2

Correlation between serum $\text{Ca}^{2+}:\text{Mg}^{2+}$ ratio and cerebrospinal fluid (CSF) $\text{Ca}^{2+}:\text{Mg}^{2+}$ ratio in healthy pregnant group.



Apostol. CSF and serum ionized Mg and Ca in preeclampsia. *Fertil Steril* 2010.

Some comments should be made as to the relationships of the various fractions of Mg measured to the principal cation-binder molecules circulating in the blood (i.e., the various protein fractions), and whether these interactions may aid in explaining a Mg-deficiency in pregnancy and untreated preeclamptic women. In 1932, Watchorn and McCance (59) demonstrated that Mg in serum could be divided by ultrafiltration into a diffusible and nondiffusible form. In these studies about 25% of the Mg in serum was nondiffusible. These investigators reported that during pregnancy, the serum Mg concentration is lowered but the ratio of ultrafiltrable to total Mg increased. This could not be explained solely by a diminished quantity of protein (59). Numerous studies have confirmed a loss of the serum proteins, albumin and gamma-globulin, in normal pregnancy (60–64). In 1970, Studd and co-workers (64–67) published a series of articles, which indicated that several proteins, in addition to albumin and gamma-globulin, are decreased in late pregnancy and more so in preeclampsia (viz., transferrin and hemopexin). All of these proteins can bind cations such as Mg and Ca. In 1981, Speich and co-workers (68), using ultrafiltration and other techniques, calculated that in healthy normal women 67% of the total Mg in serum was ionized, whereas Kroll and Elin (69), a few years later, calculated that approximately 68% of the Mg in serum was ionized. We found that 69% of the total Mg is ionized with our ion-selective electrode, in normal women, which agrees with the calculated ratios of previous workers. However, ours and other reports indicate that this fraction of ionized Mg increases in late pregnancy to about 73%–78% (8). This should be viewed in light of a loss of the major Mg- and Ca-binding proteins (60–64), which would result in elevation of the percent serum ionized Mg, as found in our study for normal late pregnancy (Table 1). Recently, several studies, using proteomics, have reported scores of other serum proteins, which were found

to be up-regulated in preeclamptic women (70, 71), thus potentially supplying additional ion-binding sites. Thus, the interesting finding, reported herein, of a reduced percentage of serum ionized Mg in Mg-treated preeclamptic women, that cannot be explained by a loss of serum albumin, transferrin, and hemopexin, but could be viewed in the light of a reported increase in other serum proteins (i.e., globulins, alpha₂-glycoproteins, alpha₂-macroglobulins, beta-lipoproteins, and various complement fractions in preeclamptic women) (64, 66, 67, 72), which can potentially bind free Mg ions.

With respect to our findings for Mg and Ca in the CSF, there is as yet no study in the literature, with evidence that CSF of mild preeclampsics contain significant amounts of protein (73).

For nearly 100 years, it has been known that preeclampsia is a placental condition (74). Placentation needs extensive angiogenesis to form a suitable framework for oxygenation and nutrition of the fetus. Therefore, numerous proangiogenic and antiangiogenic factors and proteins are elaborated by the developing placenta. Currently it is thought that placental angiogenesis is defective in preeclampsia (for review, see Ref. 73). A host of placental proteins, which gain access to the serum of preeclamptic women, have been detected (71), which may bind Mg and Ca. Thus these proteins could be an additional source for the decreased ionized Mg in the blood of preeclamptic women over and above that of normal pregnancy. Much experimentation will be required in the future to identify the precise interactions of these proteins with Mg and Ca in serum of preeclampsics.

On the other hand, the present and our previous findings, of a substantial and significant decrease in serum ionized Mg in normal pregnancy (8) would seem to indicate that, in the last analysis, it is a matter of the degree of decrease in Mg^{2+} circulating in the blood, among other factors, that controls the outcome of either a normal pregnancy or one with vascular and neurologic complications, which in extreme cases will lead to the seizures seen in eclampsia. In a study of convulsing children, the CSF Mg^{2+} was found to be significantly reduced when measured shortly after the convulsions (75). We hypothesize, therefore, that there is a critical level of serum ionized Mg, which may be patient-dependent, necessary to prevent the sequelae of preeclampsia–eclampsia. It is reasonable to suggest that a daily supplementary dose of oral magnesium should be a routine for prenatal care.

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