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Original article

Association between magnesium status, oxidative stress and inflammation in preeclampsia: A case–control study

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SUMMARY

Background & aims: Preeclampsia is responsible for more than one-third of all maternal deaths in Brazil. The objectives of the present study were to evaluate magnesium status and its association with oxidative stress and inflammation in preeclamptic women, and to identify the predictor variables of the disorder.
Methods: The study population consisted of 36 women divided into preeclamptic ($n = 18$) and control groups ($n = 18$). The preeclamptic group included women ($\geq$20 weeks of pregnancy) with arterial pressure $\geq$ 140/90 mmHg and proteinuria >0.3 g/24 h, while the control group comprised pregnant women with no clinical/obstetric complications. Magnesium intake was assessed via a food frequency questionnaire validated for pregnant women in Brazil. Plasma, erythrocyte and urinary magnesium levels were determined by flame atomic absorption spectroscopy, while oxidative stress and inflammatory markers were assessed using standard protocols. Logistic regression analysis was used to identify the predictors of preeclampsia.
Results: Preeclamptic and control groups were similar with respect to magnesium intake and urinary excretion, while plasma and erythrocyte magnesium concentrations were higher in the former group. Plasma magnesium was positively correlated with catalase and glutathione peroxidase activities and with concentrations of interleukin-6 and tumor necrosis factor alpha. Regression analysis showed that plasma magnesium and urinary 8-isoprostane were associated with preeclampsia.
Conclusion: Magnesium status appears to result from homeostatic imbalance and physiological alterations typical of preeclampsia. Increased plasma magnesium and decreased urinary 8-isoprostane were considered predictors of preeclampsia.

1. Introduction

Preeclampsia is one of the main causes of maternal and fetal mortality worldwide [1], while in Brazil, preeclampsia and eclampsia are responsible for 37% of all maternal deaths [2]. For reasons that are not fully understood, the placenta does not function properly in preeclamptic women resulting in oxidative stress, exacerbated maternal inflammation and emergence of classical symptoms such as increased arterial blood pressure and proteinuria [3].

Although the physiopathological mechanisms associated with the disorder have been studied extensively, the influence of factors such as magnesium status remains obscure. Some researchers have associated preeclampsia with the deficiency of magnesium since

Abbreviations: AUC, area under the curve; CAT, catalase; CI, confidence interval; CRP, C-reactive protein; D, difference between magnesium intake and EAR; EAR, estimated average requirement; FFQ, food frequency questionnaire; GSH-Px, glutathione peroxidase; Hb, hemoglobin; HPLC, high performance liquid chromatography; IL6, interleukin 6; MDA, malondialdehyde; NF, nuclear factor; OR, odds ratio; PCTL, percentile; RDA, recommended dietary allowance; ROC, receiver operating characteristic; ROS, reactive oxygen species; SD_D, standard deviation of D; TBA, thiobarbituric acid; TNF-α, tumor necrosis factor alpha.

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this mineral is required for numerous biochemical functions including energy production, the modulation of vascular tone and muscle contraction [4–6]. Magnesium deficiency provoked by reduced dietary intake is characterized by alterations in the compartmental distribution of the micronutrient [6], which may result in the generation of reactive oxygen species (ROS) and associated inflammation [7,8]. However, there are currently no reports concerning the relationships between magnesium status and oxidative stress, inflammation and endothelial functions in preeclampsia, although these factors appear to be critical for the development of the condition.

The objective of the present study was to test the hypothesis that magnesium deficiency aggravates oxidative stress and inflammatory response in preeclampsia. For this purpose, magnesium intake and the concentrations of the micronutrient in plasma, erythrocytes and urine were evaluated in groups of preeclamptic and healthy pregnant women, and associations between magnesium status and markers of oxidative stress and inflammatory cytokines were determined in order to identify predictor variables of preeclampsia.

2. Materials and methods

2.1. Study population

Details of the project were submitted to and approved by the Ethical Research Committees of the Hospital das Clínicas, Faculdade de Medicina and Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (protocol CAAE #1105.0.015.018-09) and authorized by the Director of Hospital Ipiranga (São Paulo, SP, Brazil). Written informed consent was obtained from all participants prior to the commencement of the study.

The sample size was calculated from the differences between means (in terms of standard deviations) of the two groups using Student *t* test, considering an effect size (Cohen's *d*) equivalent to 1 [9], assuming a statistical difference between the groups of 5% and a study power of 80%. The minimum sample size required was estimated to be 34, i.e. 17 subjects per group.

A total of 36 pregnant women (18 preeclamptic subjects and 18 healthy controls), all of whom had attended the obstetric services of Hospital Ipiranga and Hospital das Clínicas da Faculdade de Medicina (Universidade de São Paulo, São Paulo, SP, Brazil), were recruited consecutively over a period of 33 months according to predefined inclusion and exclusion criteria. For every preeclamptic individual recruited, a further healthy pregnant woman of similar gestational age was incorporated into the control group.

Preeclampsia was diagnosed on the basis of maternal blood pressure $\geq$140/90 mmHg and proteinuria >0.3 g/24 h [1] after the 20th week of pregnancy. The control group included pregnant women of similar gestational ages but with no diagnosis of preeclampsia. The exclusion criteria for both groups were women presenting chronic arterial hypertension, gestational hypertension (detected before the 20th week of pregnancy), multiple gestation, type I or type II diabetes, heart disease, smoking addiction or use of a mineral supplement containing magnesium. All participants were advised to ingest iron sulfate supplement as recommended by the Brazilian Ministry of Health.

2.2. Study design

This observational case–control study involved a group of pregnant women selected initially on the basis of information contained in medical records. At the first meeting with potential participants, the aims and objectives of the investigation were carefully explained and subjects were invited to take part in the study and to sign the informed consent. Demographic data (age, number of pregnancies and family history of preeclampsia) were collected using a questionnaire, and the information provided was verified by comparison with medical records. Participants were instructed regarding the preconditions for sampling blood after 8 h fasting and the procedure for collecting a 24-h urine sample. At the second meeting, participants were submitted to anthropometric assessment and a quantitative food frequency questionnaire (FFQ) was applied. During this meeting, urine samples collected by participants during the previous 24-h period were received and stored at −80 °C until required for analysis, and blood samples (20 mL) were obtained by venipuncture and stored in tubes with and without anticoagulant (10 μL sodium citrate/mL blood or 7.2 mg of ethylenediaminetetraacetic acid).

2.3. Anthropometric evaluation

Anthropometric measurements were performed with the subject barefoot and wearing light clothing. Body weight was determined using portable digital scales (Toledo, São Paulo, Brazil) of 250 kg capacity and 50 g sensitivity, while height was evaluated using a Toledo stadiometer, comprising a 2 m measuring tape graduated in 0.5 cm divisions, with the individual in an orthostatic position. Maternal nutritional status was based on the body mass index (BMI) for the week of pregnancy [10], as recommended by the Brazilian Ministry of Health.

2.4. Assessment of magnesium intake

An FFQ, validated for use with pregnant women in Brazil [11], was applied by a trained nutritionist and the data were analyzed using the on-line NutriQuanti system [12]. Magnesium intake was assessed according to dietary reference intakes [13], while the probability of nutrient inadequacy was determined by calculating the difference (D) between the observed intake of the individual and the estimated average requirement according to age and physiological status. The standard deviation of D (SD_D) was calculated and the D/SD_D ratio used to determine the probability of correctly concluding that the magnesium intake value of an individual was adequate or inadequate in relation to the mean reference value [14].

2.5. Biochemical analyses

Quantitative determinations of the levels of magnesium in serum, plasma and urine were performed using a flame atomic absorption spectrometer (AAnalyst 100, Perkin–Elmer, Norwalk, CT, USA). All glassware and tools were subjected to demineralization with 30% nitric acid for 12 h prior to use in assays. In order to reduce potential interference by aluminum, a solution of 5% lanthanum oxide was added to all samples, including those employed in constructing the calibration curve, to give a final concentration of lanthanum of 0.1% [15]. Standard reference samples (Seronorm™ Trace Elements Serum L-1 and Urine Blank, Sero, Billingstad, Norway), together with secondary reference samples (plasma, erythrocyte and urine pools), were used to determine the performance of the analytical procedures, revealing an accuracy of 101% and a precision between 84 and 99%.

Plasma malondialdehyde (MDA) concentration, taken as a measure of lipid peroxidation, was assessed by high performance liquid chromatography (HPLC) performed using a Shimadzu (Kyoto, Japan) instrument fitted with a reverse-phase C_{18} column (150 × 4.6 mm; 5 μm particle size; Phenomenex, Torrance, CA). Reaction between MDA and thiobarbituric acid (TBA) formed the $MDA\text{-}TBA_2$ chromogen, the absorbance of which was monitored at