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Strontium and its role in preeclampsia

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ABSTRACT

Background: Preeclampsia (PE) is considered a specific vascular disease in which endothelial dysfunction may be the crucial factor of its pathogenesis. It has been suggested that strontium (Sr) may play a role in the pathophysiology of PE. Our group established in a previous study the serum levels of Sr in healthy pregnancies, and the main aim of the present study was to evaluate Sr concentrations and oxidative status in preeclamptic women.
Methods: The study population included women with early-onset PE (E-PE, n = 39), late-onset PE (L-PE, n = 67) and serial samples from a subset of preeclamptic women (PE-ss, n = 20). The control group included women with gestational hypertension (GH, n = 56) and healthy pregnancies (samples collected in the 1st (n = 50), 2nd (n = 51) and 3rd trimesters (n = 53)). Strontium, calcium (Ca), uric acid (UA), placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1), N-terminal pro-brain natriuretic peptide (NT-proBNP), lipid peroxidation and total antioxidant activity (TAA) were measured in these samples.
Results: Mean Sr levels were significantly higher in PE than in control groups ($p \leq 0.0001$). Calcium values were found to be significantly lower in E-PE compared to control groups ($p = 0.03$). Higher levels of NT-proBNP were found in PE vs. control groups ($p < 0.001$). sFlt-1/PlGF ratio was higher in E-PE compared to L-PE and GH ($p < 0.001$). Uric acid levels in PE were significantly higher than in control groups ($p < 0.0001$). There was a strong positive correlation between UA and Sr in the E-PE serial samples ($r = 0.80$, $p < 0.0001$). Lipid peroxidation and lipid peroxidation/TAA ratios were found to be higher in PE, with lower values of TAA.
Conclusion: The higher levels of Sr and the alterations of redox status found in preeclamptic women, along with the strong correlation between UA and Sr suggest that this element may be involved in the pathogenesis of PE.

1. Introduction

Endothelial dysfunction contributes significantly to the pathogenesis of preeclampsia (PE) and has been related to the oxidative stress of placenta [1–7]. Oxidative stress is manifested at the maternal–fetal interface from early pregnancy onwards [4], but in the case of PE, an imbalance between the enzymatic antioxidant activity and the formation of lipid peroxides takes place [5,8–11]. Thus, trace elements involved in the antioxidant system, such as copper, selenium and zinc, have been studied in preeclamptic pregnancies [11–14], yielding contradictory results and, therefore, failing to reach a consensus regarding the relationship between the deficiency/excess of these elements and PE. One of these trace elements, strontium (Sr), has recently been associated with the pathophysiology of PE [15]. In addition, strontium ranelate, a medication for osteoporosis, has been linked to an increased risk of cardiovascular and thromboembolic events [16,17].

Since PE is considered a specific vascular disease, in which endothelial dysfunction may be the crucial factor of its pathogenesis [18], and taking into account the above, we speculated that Sr may be involved in the pathogenesis of PE. The aims of this study were: i) to evaluate the serum concentrations of Sr in patients with early- and late-onset PE and compare them with the levels in non-preeclamptic

Abbreviations: 1TC, 1st trimester control group; 1T non-PE, 1st trimester of non-preeclamptic women; 1T-PE, 1st trimester of preeclamptic women; 2TC, 2nd trimester control group; 3TC, 3rd trimester control group; Ca, calcium; E-PE, early-onset preeclampsia; GH, gestational hypertension; HUCA, Hospital Universitario Central de Asturias; ICP-MS, inductively coupled plasma-mass spectrometry; L-PE, late-onset preeclampsia; MDA, malondialdehyde; NT-proBNP, N-terminal pro-brain natriuretic peptide; PE, preeclampsia; PE-ss, serial samples of preeclamptic women; PlGF, placental growth factor; ROC, receiver operating characteristic; sFlt-1, soluble fms-like tyrosine kinase 1; Sr, strontium; TAA, total antioxidant activity; UA, uric acid

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pregnant women; ii) to determine the diagnostic value of Sr; iii) to assess the cardioventricular function through measurement of N-terminal pro-brain natriuretic peptide (NT-proBNP) serum levels; iv) to determine the concentrations of other analytes (calcium (Ca), uric acid (UA), soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF)), to elucidate their possible relation with Sr and v) to evaluate and compare the redox status in a subset of patients and healthy controls and determine its association with Sr concentrations.

2. Material and methods

2.1. Study population

A total of 162 participants were enrolled in the study between October 2012 and June 2016. Serum samples from pregnant women with suspected PE diagnosis were collected at the Emergency Room of Hospital Universitario Central de Asturias (HUCA) and analyzed at the Laboratory of Medicine (HUCA). Diagnosis of PE was made according to the classical criteria: onset of hypertension and proteinuria after 20 weeks of gestation [19], being considered early-onset PE before gestational week 34 and late-onset PE after 34 gestational weeks. The study population was split in three groups, depending on the final diagnosis: early-onset PE (E-PE, n = 39), late-onset PE (L-PE, n = 67) and gestational hypertension (GH, n = 56), considering the latter as part of the control group. When several samples were collected from the same patient, the analyzed sample was the closest to the time of delivery. Whenever it was available, first trimester samples were recovered from a Biobank composed of specimens used in HUCA's First Trimester Combined Screening Program for Fetal Aneuploidies. Twenty pre-eclamptic women who had several samples collected throughout the pregnancy were selected (8 E-PE and 12 L-PE), forming an additional PE group of serial samples (PE-ss).

The control group was formed by 56 pregnant women with gestational hypertension previously mentioned and 154 healthy pregnant women whose samples were collected in the 1st (1TC, n = 50), 2nd (2TC, n = 51) and 3rd gestational trimesters (3TC, n = 53), and analyzed in our previous study [20]. Clinical data from all participants, including maternal age, gestational week of sampling and delivery and type of delivery were collected from their medical records by using the software products Cerner Millennium® and GestLab®. The study population, including the control group from our previous study [20], is shown in Fig. 1.

2.2. Exclusion criteria

Participants with multiple gestation (n = 29) and whose sample was collected post-partum (n = 2) were excluded from the study. In addition, one participant younger than 18 years old was also excluded.

2.3. Collection of serum samples

Samples were collected in BD Vacutainer® red top tubes with no additives. Serum was obtained by centrifugation at 3000 rpm for 10 min at 4 °C, and UA, PlGF, sFlt-1 and NT-proBNP were measured at the Emergency Biochemistry Laboratory of HUCA. After that, serum was aliquoted and stored at −40 °C until assayed for the rest of parameters.

In all these samples, Sr and Ca were also measured. A total of 40 serum samples from different groups (10 from E-PE, 10 from L-PE, 10 from GH and 10 collected at the 1st trimester of preeclamptic women) were randomly selected to evaluate redox status, by measuring lipid peroxidation and total antioxidant activity (TAA) at the Department of Morphology and Cell Biology, Faculty of Medicine, University of Oviedo. Likewise, 20 samples from the previous study [20] were randomly selected and recovered (10 from 1TC and 10 from 3TC) to perform the same redox status assessment.

Some analytes could not be measured in all samples due to insufficient volume. The analytes and samples assayed are shown in Supplementary data, Table S1.

2.4. ICP-MS method and sample preparation

An alkaline working solution with 1% ammonium hydroxide, 0.05% EDTA, 0.05% Triton X-100, 4% butan-1-ol and 20 µg/L of internal standard (^{72}Ge) was first prepared. The pH of this solution was 10.7. Serum samples were diluted 1:20 with this solution prior to analysis. The limit of quantification and calibration range for Sr (6-point calibration) were 3.25 (0–60) µg/L. The integration time was 2 s for ^{88}Sr and 0.5 s for ^{72}Ge. The total acquisition time was 14 s, measurements were performed in triplicate using an ICP-MS 7700 x from Agilent Technologies® and total analysis time was less than three minutes per sample. In the collision cell, the helium flow was on (flow rate of 4.5 ml/min). The octopole voltages were: Bias = −18.0 V, RF = 200 V, and the energy discrimination was set at 3.0 V.

2.5. Spectrophotometry and electrochemiluminescence immunoassay methods

Calcium and UA levels were determined on the automated auto-analyzer Cobas c601 (Roche Diagnostics, Germany) by a spectrophotometric assay (Elecsys®). The measuring intervals were 0.2–5 mmol/L and 0.2–25 mg/dL for Ca and UA, respectively. The coefficients of variation were ≤2.5% and ≤1.6% for Ca and UA, respectively. The lower detection limits for Ca and UA were 0.2 mmol/L and 0.2 mg/dL, respectively.

PlGF, sFlt-1 and NT-proBNP concentrations were measured by an electrochemiluminescence immunoassay (ECLIA, Elecsys®) on the automated immunoanalyzer Cobas e601 (Roche Diagnostics, Germany). The measuring intervals were 3–10000 pg/mL, 10–85000 pg/mL and 5–35000 pg/mL for PlGF, sFlt-1 and NT-proBNP, respectively. The coefficients of variation were ≤4.6%, ≤5.6% and ≤3.1% for PIGF, sFlt-1 and NT-proBNP, respectively. The lower detection limits for PIGF, sFlt-1 and NT-proBNP were 3, 10 and 5 pg/mL, respectively.

2.6. Redox status determination

The lipid peroxidation was used as an indicator of oxidative damage. This process leads to the production of lipid peroxides and its derivative end-products malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) that provide a convenient rate of lipid peroxidation [21]. MDA and 4-HNE content was determined using the 1-methyl-2-phenylindole method, based on the condensation of the chromogenic agent 1-methyl-2-phenylindole with either MDA or 4-HNE [22]. The results are expressed as nmol MDA + 4-HNE/g protein.

The total antioxidant activity was determined using the ABTS (2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)) cation radical method [23] modified for blood samples [24]. Results are expressed in equivalents of mg Trolox/g protein.

2.7. Statistical analysis

Statistical analyses were performed with MedCalc® 12.5.0. Continuous variables were expressed as means and standard deviations whereas discrete variables were expressed as numbers or percentages. Normal distribution was evaluated using the Kolmogorov-Smirnov test. When the data did not follow a normal distribution (even using the logarithmic transformation), the variables were expressed as median and interquartile range. Analysis of variance test was used to compare various groups with independent samples. Student's *t*-tests were used for comparison between two groups of independent and paired samples. The Welch test was used for independent samples with unequal variances. Mann-Whitney tests were used when any of the groups compared did not follow a normal distribution. Receiver operating