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Oxidative stress markers and thrombomodulin plasma levels in women with early and late severe preeclampsia

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ABSTRACT

Background: Preeclampsia (PE) is a pregnancy disease associated with oxidative stress and endothelial dysfunction. It can be classified according to the severity and onset-time of clinical symptoms (early PE: < 34 weeks, late PE: ≥ 34 weeks).

Methods: We evaluated markers of oxidative stress (thiobarbituric acid reactive substances-TBARs and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-MTT) and endothelial lesion (thrombomodulin-TM) in early (N = 24) and late severe PE(N = 22) and normotensive pregnant women(N = 26).

Results: MTT levels were higher in early sPE than in normotensive pregnancy (P = 0.03). No difference was found comparing late sPE versus normotensive pregnancy, and early sPE versus late sPE. TM levels were higher in early sPE comparing to late sPE women (P = 0.05), but no difference was found between early or late sPE versus normotensive groups. TBARs levels did not differ significantly among the three groups. These data suggest that endothelial lesion and the antioxidant status are more pronounced in early sPE. Moreover, lipid peroxidation might be an early event in PE, stimulating a compensatory antioxidant defense later in pregnancy.

Conclusions: Longitudinal studies involving pregnant women with risk factors for PE development and including other methods for oxidative stress and endothelial lesion determination should be conducted in order to better evaluate the role of these processes in PE pathogenesis.

1. Introduction

Preeclampsia (PE) is a pregnancy disease characterized by new onset hypertension and multiple organ dysfunctions, associated or not with proteinuria, that manifest clinically after 20 weeks of gestation [1]. PE has been classified in early and late forms, according to the onset of clinical symptoms (< 34 weeks or ≥34 weeks, respectively) [2]. Late PE is more prevalent than early PE and mostly associated with maternal risk factors. By contrast, early PE is usually more severe and involves placental abnormalities [3].

It is widely accepted that defective extravillous trophoblast invasion toward uterine spiral arteries in early pregnancy lead to ischemia/reperfusion injury due to high generation of reactive oxygen species (ROS) [3], resulting in villous and vascular lesions in the placenta [4]. Membrane lipid peroxidation, endothelial cell dysfunction and systemic inflammation may be consequences of placental oxidative stress in PE women. In fact, there is a crosstalk between these processes, i.e., one biological alteration may induce or exacerbate another [5,6].

Results from Gupta et al. indicated higher concentration of oxidative stress markers, such as thiobarbituric acid reactive substances (TBARs), and lower antioxidant defenses, like vitamins C and E, in biological samples from PE women when compared with normotensive pregnant controls [7]. However, some antioxidant systems have been shown to be up-regulated or unchanged during PE [8]. The antioxidant status of plasma samples can be investigated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay. A previous study revealed higher plasma levels of MTT in PE women compared to normotensive pregnant women [9].

Thrombomodulin (TM) is a transmembrane glycoprotein expressed in endothelial cells that acts as a receptor for thrombin [10]. Full-length TM can be cleaved by inflammatory proteases and oxygen radicals, releasing its soluble form into the circulation, which is regarded as a reliable marker of endothelial cell injury [11,12]. Our group and others found increased levels of soluble TM in the circulation of PE women [13–15].

Despite the aforementioned putative relationships between

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oxidative stress and endothelial dysfunction in PE, the exact molecular mechanisms in which these processes contribute to the disease pathogenesis remain unclear.

2. Material and methods

2.1. Study participants

This case control study included 72 Brazilian women in the third trimester of gestation, who were distributed in three groups: normotensive pregnancy (NP, N = 26), early severe PE (sPE) (N = 24) and late sPE (N = 22). Ethics approval was obtained from Universidade Federal de Minas Gerais (Institutional Review Board) and all participants signed the written informed consent. This research was carried out according to the Declaration of Helsinki, as revised in 2008. The participants were both recruited and their blood samples collected at the time of hospital admission for PE women and at a routine clinic visit for normotensive pregnant women. Participants were not followed beyond this single timepoint.

sPE was defined by blood pressure ≥ 160/110 mmHg after rest, at least in 2 occasions, ≥4 h apart, after 20 weeks of gestation. Other clinical signs and symptoms of sPE included new onset cerebral or visual disturbances, progressive renal insufficiency and impaired liver function [1]. Pregnant women with sPE were stratified in early sPE and late sPE according to gestational age of clinical symptoms onset (< 34 or ≥34 weeks, respectively) [2]. The normotensive pregnant group included healthy women with no history of hypertension. Exclusion criteria common for all groups were: chronic hypertension, diabetes mellitus, cancer, coagulation disorders; cardiovascular, autoimmune, hepatic, renal and inflammatory/infectious diseases. Clinical and laboratory data were obtained through search on medical records and interviews with the participants.

2.2. Blood sampling and processing

Peripheral blood samples were collected into sterile tubes containing sodium citrate and centrifuged at 3500 ×*g* for 15 min at room temperature. The plasma aliquots were stored at −80 °C until assayed.

2.3. Measurement of TBARs, MTT and TM plasma levels

Lipid peroxidation was assessed through TBARs assay. The total antioxidant activity of the samples was investigated through MTT assay. TBARs (1) and MTT (2) assays were performed according to the following protocols, as summarized: (1) A mixture containing thiobarbituric acid 1%, sodium hydroxide 0.05 mol/l, butylated hydroxytoluene 0.1 mmol/l and concentrated phosphoric acid was added to the plasma samples, which were incubated in the dry bath at 98 °C for 25 min and then put in the freezer for 10 min. Subsequently, butanol was added to the tubes, which were vortexed and centrifuged at 2000 ×*g* for 5 min. The absorbance of the supernatant was measured in a spectrophotometer at 532 and 600 nm. The concentration of TBARS was calculated using the molar extinction coefficient of 156/(mmol/l × cm) [16]. TBARs values are expressed as μmol/l of plasma; (2) A mixture containing the plasma sample, phosphate buffered saline (PBS) 0.1 mol/l and MTT solution (0,5% in PBS) was incubated protected against light at 37 °C for 60 min. Then, a solution of isopropanol:HCl was added and the samples were vortexed and centrifuged at 3000 ×*g* for 10 min. The absorbance of the supernatant was measured in a spectrophotometer at an optical density (O.D.) of 570 nm [17].

A commercially available enzyme-linked immunosorbent assay (Quantikine ELISA kit for human Thrombomodulin/BDCA-3, R&D Systems) was used to measure TM plasma levels, according to the manufacturer's instructions. Plasma TM values are expressed as pg/ml.

2.4. Assessment of laboratory parameters

The possible correlations among the biomarkers measured in this study (TBARs, MTT and TM), biochemical and hematological parameters that have been traditionally correlated with clinical outcome in PE were also investigated. The biochemical parameters considered were 24 h proteinuria and the plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine, uric acid, and total bilirubin; and the hematological parameters were hemoglobin and hematocrit levels, red blood cells count (RBC), white blood cells count (WBC) and platelets count. These data were available only for sPE women. The normal ranges considered in this study were based on a population of healthy pregnant women in the third trimester of gestation [1,18], as follows: proteinuria (< 0.3 g/24 h urine specimen), AST (4–32U/l), ALT (2–25U/l), LDH (82–524U/l), creatinine (0.4–0.9 mg/dl), uric acid (3.1–6.3 mg/dl), total bilirubin (0.1–1.1 mg/dl), hemoglobin (9.5–15.0 g/dl), hematocrit (28.0–40.0%), RBC (2.71–4.43 × 10^6/mm^3), WBC (5.9–16.9 × 10^3/mm^3) and platelets count (146–429 × 10^3/mm^3).

2.5. Statistical analysis

Statistical analysis was performed using *Statistica* software for Windows. The normality of continuous variables was assessed by Shapiro-Wilk's W-test. The comparison of variables with normal distribution was performed by ANOVA test with Tukey's test (three groups) or Student's *t*-test (two groups) and is presented as mean ± standard deviation (SD). Continuous variables that did not follow a normal distribution were analyzed by Kruskal-Wallis test followed by Ranks' test (three groups) or Mann-whitney U test (two groups) and are presented as median (minimum - maximum values). Correlation analysis among the biomarkers (TBARS, MTT and TM) and the biochemical and hematological parameters were evaluated by Spearman coefficients (Rs). A P value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the studied pregnant women are described in Table 1. There were no significant differences comparing age, body mass index (BMI) and gestational weight gain (GWG) until the moment of sampling among the three studied groups. Gestational age of sampling differed significantly among the three groups (all P = 0.0001) and it was higher in late sPE women than in early sPE women, as expected. Both systolic and diastolic blood pressures were significantly higher in early sPE and in late sPE than in the normotensive pregnant women (NP) (P = 0.0001 in both cases). There was no significant difference in systolic and diastolic blood pressures between early sPE and late sPE.

3.2. Laboratory parameters

The mean or median values of all laboratory parameters from early and late sPE women were within the normal ranges considered for normotensive pregnant women in the third trimester of pregnancy [18]. None of them differ significantly between early and late sPE, although uric acid levels tended to be increased in the sPE group (P = 0.058) (Table 2).

3.3. TBARs, MTT and TM plasma levels

Early sPE women [0.12 (0.01–0.25) O.D. 570 nm] showed higher plasma levels of MTT than normotensive pregnant women (NP) [0.10 (0.07–0.15) O.D. 570 nm] (P = 0.03). No difference was found in MTT levels when comparing late sPE women [0.11 (0.08–0.19) O.D. 570 nm]