



Original

Enzyme activity of superoxide dismutase and zincemia in women with preeclampsia

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Abstract

Introduction: Preeclampsia is a complex syndrome of unknown aetiologic origin. It is characterized by the clinical triad of hypertension, edema and proteinuria. Current concepts of the genesis of preeclampsia include endothelial dysfunction and oxidative stress.

Objective: This study assessed the relationship between the activity of superoxide dismutase, the zincemia and the preeclampsia.

Methods: A case-control study was carried out with 94 women, age between 17 and 44 years, which were divided in two groups: women with preeclampsia (n = 44) and control group women with normal pregnancy (n = 50). The activity of superoxide dismutase enzyme was determined according to Ransod kit. Zinc concentrations in plasma and erythrocytes were determined using the flame atomic absorption spectrophotometry technique, both determined before delivery and during puerperium. Student's t-test and the paired t-test were used for statistical analysis, and significance was established at $p < 0.05$.

Results: The study revealed plasma hypozincemia more pronounced in women with severe preeclampsia ($p < 0.05$). This behavior was not observed in erythrocytes, whose concentrations remained stable, before delivery and puerperium in women with preeclampsia and normal pregnancy. The antioxidant activity of the enzyme superoxide dismutase was found elevated in women with and without preeclampsia ($p > 0.05$), with significant reduction in activity in the postpartum period ($p < 0.05$) in both groups studied ($p > 0.05$).

Conclusions: This study shows there are alterations in biochemical parameters of zinc in women with preeclampsia. The activity of superoxide dismutase shows that this marker cannot be used to predict the evolution of the disease.

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Key words: Preeclampsia. Oxidative stress. Superoxide dismutase. Zinc.

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LA ACTIVIDAD DE LA ENZIMA SUPERÓXIDO DISMUTASA Y ZINCEMIA EN MUJERES CON PREECLAMPSIA

Resumen

Introducción: La preeclampsia es un síndrome de origen desconocido del punto de vista etiológico. Se caracteriza clínicamente por una tríada de síntomas: edema, hipertensión y proteinuria. Los conceptos actuales sobre la patogénesis de la preeclampsia incluyen la disfunción endotelial y el estrés oxidativo.

Objetivo: En este estudio se evaluó la relación entre la actividad de la superóxido dismutasa, zincemia y preeclampsia.

Métodos: Este estudio de casos y controles se llevó a cabo con 94 mujeres, con un rango de edad situado entre 17 y 44 años, los que fueron divididos en dos grupos: mujeres con preeclampsia (n = 44) y grupo control, las mujeres con embarazo normal (n = 50). La actividad de la superóxido dismutasa se llevó a cabo según el kit Ransod. Las concentraciones de zinc en plasma y los eritrocitos fue realizada según el método de espectrofotometría de absorción atómica de llama, ambos determinados antes del nacimiento y después del parto. La prueba de la t de Student y la prueba t pareada fue utilizada para el análisis estadístico ($p < 0,05$).

Resultados: El estudio reveló hypozincemia en el plasma, con valores más altos en las mujeres con preeclampsia severa ($p < 0,05$). Esta situación no fue observada en los eritrocitos, cuya concentración se mantuvo estable antes del parto y en el puerperio, en las mujeres con preeclampsia y en las con embarazo normal. La actividad antioxidante de la superóxido dismutasa, se encontró elevada en las mujeres con y sin preeclampsia ($p > 0,05$), con una reducción significativa en la actividad en el periodo postparto ($p < 0,05$) en ambos grupos ($p > 0,05$).

Conclusiones: Este estudio revelló que hay cambios en los parámetros bioquímicos de zinc en mujeres con preeclampsia. La actividad de la superóxido dismutasa se revelló que este marcador no se puede utilizar para predecir la progresión de la enfermedad.

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Palabras clave: Preeclampsia. Estrés oxidativo. Superóxido dismutasa. Zinc.

Abbreviations

Cu/Zn-SOD: Copper and zinc dependent superoxide dismutase.

SOD: Superoxide dismutase.

MDER: Dona Evangelina Rosa Maternity Hospital.

ACOG: American College of Obstetricians and Gynecologists.

Hb: Hemoglobin.

ROS: Reactive oxygen species.

PE: Pre-eclamptic.

BD: Before delivery.

PD: Post-delivery.

Introduction

Preeclampsia is a condition that is unique to human pregnancy and is characterized by the clinical triad of hypertension, edema and proteinuria.¹ It has a prevalence of 2 to 10% of pregnancies worldwide and it is a major cause of the increase in maternal and perinatal morbidity and mortality, which in Brazil accounts for 37% of direct obstetric causes of death.^{2,3}

However, the etiology of preeclampsia remains unknown. It is believed that the maternal organism adapts poorly to pregnancy due to a combination of genetic, immunological and environmental factors. A defective trophoblastic invasion of spiral arterioles causes hypoxia in the placenta with the release of factors that determine endothelial dysfunction with increased vascular resistance.^{4,5}

Two factors have been considered important in the pathophysiological mechanism of this syndrome: lipid peroxidation and the exacerbated inflammatory response.^{6,7} The membrane is one of the cellular components most affected by lipid peroxidation. Its structure and permeability change and cytotoxic products are produced.⁸ Therefore, in aerobic systems, it is essential that there is a balance between oxidoreducing agents, such as the reactive species of oxygen metabolism, and the antioxidant defense system, which acts as a detoxifying agent before free radicals cause cellular injury.⁹

Copper and zinc dependent superoxide dismutase (Cu/Zn-SOD) is an enzyme that acts by catalyzing the dismutation reaction of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and O_2 in the presence of the proton H^+ and it is involved in cellular protection against the toxicity caused by reactive oxygen species.^{9,10} The participation of minerals such as copper, zinc, selenium, magnesium and calcium has been investigated in the development of preeclampsia.¹²⁻¹⁴ Zinc is part of the structure of superoxide dismutase (SOD), and this enzyme's activity may decrease if there is a zinc deficiency.¹¹

Many studies on the etiology and complications of preeclampsia suggest that there is an exacerbated release of free radicals and an abnormally low response

of antioxidants, which characterizes oxidative stress,¹⁵⁻¹⁷ contributing to endothelial dysfunction leading to preeclampsia. Therefore, the aim of this study was to measure the activity of the enzyme SOD, zincemia and the relationship between these parameters and preeclampsia.

Patients and methods

The case-control study was carried out at Dona Evangelina Rosa Maternity Hospital (MDER) in Teresina, PI, Brazil, from July 2009 to January 2010. The study included pregnant women, during their prepartum period, who were hospitalized at the Intensive Care Unit in the obstetric wards and obstetric and surgical centers.

Women smokers, with diabetes mellitus, essential hypertension, chronic renal failure, thyroid disorders, lupus erythematosus, urinary tract infection and cervical/vaginal inflammation were excluded from the study.

For the diagnosis of preeclampsia, the study adopted the criteria of the "American College of Obstetricians and Gynecologists (ACOG) 2002".¹⁸ Blood pressure was measured in a sitting position, considering the 5th Korotkoff sound. Afterwards, proteinuria was determined using a measuring tape, on a scale from 1 to 4 crosses, in a single sample.

The project was approved by the Ethics Committee of the Federal University of Piauí, and Dona Evangelina Rosa Maternity Hospital, according to the protocol (CAAE 0133.0.045.000-08). The participants gave written consent.

Blood samples (15 ml) were taken in the morning, after fasting for at least 12 hours. The first collection took place in the prepartum period, on the day of pregnancy resolution, and the second 30-45 days after delivery. From the total blood collected, 5 ml were distributed in test tubes with EDTA to determine SOD activity, and 10 ml were placed into a tube containing sodium citrate at 30% as an anticoagulant to analyze the zinc content.

The plasma was separated from whole blood by centrifugation at 1,831x g for 15 minutes at 4° C (SIGMA 4K15 centrifuge), and stored at -20° C. The blood sediment was rinsed with normal saline at 0.9%, centrifuged at 2,493 x g for 10 minutes, and the supernatant was discarded. This procedure was carried out three times to remove contaminants from the erythrocyte (platelets and leukocytes). After the last centrifugation, the saline was aspirated and discarded, and the erythrocytary mass was extracted with the aid of an automatic pipette, transferred to polypropylene tubes and kept at -80° C to determine the activity of SOD, and at -20° C for the analysis of zinc.

The activity of SOD enzyme in erythrocytes was determined by the *in vitro* method, according to methodology recommended by the manufacturer Randox. In

Table I
Blood pressure of patients with preeclampsia and control group

Variables	Control n = 50	Preeclampsia n = 44
Systolic blood pressure (mm Hg)-BD	116.6 ± 8.6	158.4 ± 16.8*
Systolic blood pressure (mm Hg)-PD	107.5 ± 6.7	119.8 ± 12.9***
Diastolic blood pressure (mm Hg)-BD	74.2 ± 7.7	104.8 ± 12.4*
Diastolic blood pressure (mm Hg)-PD	66.0 ± 6.4	73.7 ± 9.8***

Values are expressed as mean ± SD.

BD: Before delivery; PD: Post-delivery.

*p < 0.0001 preeclampsia versus control.

**p < 0.05 when compared to before delivery.

addition to the sample, the following were prepared: the substrate mixture, xanthine oxidase buffer and standard buffer for subsequent calculation of enzyme activity, with results expressed as U/Hb. The reference values are 1,102-1,601 U/gHb according to the Ransod/Randox Kit.

Plasma zinc levels were measured by atomic absorption spectrophotometry according to the method proposed by Rodriguez et al.¹⁹. Two aliquots were taken from each plasma sample, diluted in Milli-Q® water at 1:4 and aspirated directly on the flame of the device. Titrisol® (Merck), diluted in Milli-Q® water with 3% glycerol, was used as standard at concentrations of 0.1, 0.2, 0.3, 0.5 and 1.0 µg/ml. Erythrocyte zinc levels were measured by atomic absorption spectrophotometry (Whitehouse et al.).²⁰ The reference values for plasmatic zinc were 70-110 µg/dL-Gibson, 1990²¹, and for erythrocytary zinc were 40-44 µg/Hb-Guthrie; Piciano, 1994.²² Hemoglobin was measured using the cyanmethaemoglobin method.²³ Results were expressed as mgZn/gHb.

In the pre and postpartum periods, the variables of the same group were analyzed using the paired t test, and in different groups, in the same period, using the independent Student t test. The difference was considered significant when p < 0.05. Data was analyzed using the Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

The mean age of the pregnant women, in years, was significantly higher for the control group, 24.13 ± 6.43, than the preeclampsia group, 27.00 ± 6.59 (p = 0.029). The mean gestational age, in weeks, was higher (p < 0.001) for patients in the control group, 39.17 ± 1.76, than in the preeclampsia group, 36.30 ± 3.01.

The table I shows the values of the blood pressure of patients for the two groups. There was statistically significant difference in value of systolic pressure (p < 0.001), which was higher in the preeclampsia group, when compared with the control, before and after

Table II
Activity of the superoxide dismutase enzyme in pregnant women of the control and preeclampsia groups

	SOD (U/gHb)	
	BD	PD [#]
Control n = 50	3,436.8 ± 5.0	2,847.6 ± 765.7
Mild PE n = 20	3,157.2 ± 677.4	2,871.8 ± 827.2
Severe PE n = 24	3,333.4 ± 689.8	2,435.9 ± 622.7

Values are expressed as mean ± SD.

PE: Pre-eclamptic; BD: Before delivery; PD: Post-delivery.

[#]p < 0.05 when compared to before delivery.

childbirth. Similar behavior was also found for the diastolic pressure. In the puerperium, there was a significant reduction of systolic and diastolic pressure when compared with the prepartum period, in the preeclampsia group.

The mean values of activity SOD were high, in both the control and preeclampsia group, without statistical difference (p > 0.05). Conversely, enzyme activity decreased significantly (p < 0.001) when values were compared between the prepartum and postpartum (table II).

In the prepartum, the levels of plasmatic zinc concentration decreased significantly (p < 0.05), when compared to the minimum reference value (70.0 µg/dL). In the postpartum period, the mean concentration increased significantly (p < 0.001), tending to normality, although there was no significant difference between the control and preeclampsia group, mild and severe.

Conversely, during the prepartum, the mean concentration of erythrocytary zinc was higher than the reference values (p > 0.05). In the postpartum period, the levels of zinc in this cellular compartment remained stable, without difference between the groups (table III).

Discussion

The results of the study showed that activity of SOD was higher in the women of the two study groups, when

Table III
Plasmatic and erythrocyte concentration of zinc in controls and pregnant with preeclampsia

Variables	Zn plasm (µg/dL)		Zn erythrocytes (µg/gHb)	
	BD	PD [#]	BD	PD
Control n = 50	48.3 ± 8.3	58.9 ± 8.6	53.2 ± 10.2	47.4 ± 12.8
Mild PE n = 20	50.0 ± 9.4	60.8 ± 7.8	48.8 ± 9.2	47.5 ± 12.2
Severe PE n = 24	38.8 ± 8.2***	57.0 ± 10.5	47.3 ± 8.9	49.2 ± 13.2

Values are expressed as mean ± SD.

PE: Pre-eclamptic; BD: Before delivery; PD: Post-delivery.

*p < 0.05 when compared to control group.

**p < 0.05 when compared to mild pre-eclamptic group.

[#]p < 0.05 when compared to before delivery.

compared to reference values for adults, with more pronounced activity in pregnant women in the control group, but without statistical significance.

Our results are in accordance with those of Dirican et al.,²⁴ who evaluated the antioxidant status in preeclampsia, and found no significant difference in erythrocytary SOD activity between the group with the disease and the control. The literature suggests that increased lipid peroxidation is the body's natural response to pregnancy when pregnant women with and without preeclampsia are compared with non-pregnant women. Other studies suggest that high levels of SOD activity in normal pregnant women are indicative of the body's ability to compensate for the increasing state of injury caused by reactive oxygen species.^{16,25,26}

The similar activity of SOD found in the two groups of pregnant women differs to the data in the literature, which reports significant reductions in erythrocyte, plasma, whole blood and umbilical cord blood samples SOD activity in women with this disease, when compared to normal pregnant women.^{13,15-17,25}

On the other hand, Llurba et al.²⁷ and Sharma et al.²⁸ found activity of SOD that was significantly higher in women with preeclampsia, especially in those with more severe disease, when compared to those with normal pregnancy. Llurba et al.²⁷ believe that the increase in enzyme activity is a compensatory and adaptive effect to the high levels of reactive oxygen species (ROS). It is thought that the main way that cells counteract free radical damage is by the increased expression of Cu/Zn-SOD. In preeclampsia, this mechanism might protect against the initial process of peroxidation. However, the antioxidant capacity in pre-eclamptic patients does not reach a magnitude sufficient to eliminate oxidative stress.²⁹

In the postpartum period, the reduction in activity of SOD found in the women of the control and preeclampsia groups, suggests that the placenta plays an important role in the production of reactive oxygen species. These results are supported by literature that shows there is a decrease of lipid peroxidation products after delivery.^{16,26} This characterizes a reduction in oxidative stress and a lower antioxidant response.

Our results contrast with those of Chamy et al.,¹⁶ who evaluated the activity of SOD in plasma, and found higher values in patients with and without preeclampsia, 30 days after delivery, with a statistical difference between groups. The healthy pregnant women returned to normal values of enzyme activity 30 days after delivery, while those with preeclampsia only returned to normal 120 days after delivery.

Regarding the study of zincemia in the women surveyed, the results revealed that the zinc concentrations in the plasma were below the normal reference values, with a significant difference between the groups of pregnant women with mild preeclampsia and severe preeclampsia, and between the severe and control group. Similar results were found by Kurum et al.¹⁴ and Jain et al.¹² who studied serum zinc and found

significantly lower concentrations of the mineral in patients with preeclampsia when compared to the control group. Atamer et al.¹³ also researching zinc in serum, found significantly lower levels of the mineral in women with and without preeclampsia, compared to non-pregnant women, with no difference between the preeclampsia group and healthy pregnant women.

During pregnancy, the plasmatic zinc decreased by 20% to 30% from the third month, decreasing with the progress of pregnancy. Hypozincemia is related to hemodilution, increased urinary excretion and the transfer of this mineral from mother to the growing fetus. Another important aspect in the reduction of this mineral in the plasma is the redistribution of the fluid compartment into the erythrocytes, by the increase of erythrocytary carbonic anhydrase and SOD activity – zinc dependent enzymes. High levels of corticosteroids are also related to this common reduction at the end of a normal pregnancy.^{30,31} In pregnant women with preeclampsia, low serum zinc may be partly due to reduced concentrations of transport proteins and estrogen caused by increased lipid peroxidation.¹⁴

The high concentration of zinc in the erythrocytes demonstrates a satisfactory nutritional status for the mineral. The erythrocytary zinc is a parameter of long-term nutritional status, because its levels only change in cases of severe deficiency.²¹

The study found that there was high activity of SOD in all pregnant women, which indicates a strong antioxidant defense during pregnancy. Therefore, this is a non-specific marker for the assessment of the disease's evolution. The reduced plasmatic zinc concentrations of pregnant women in the control and preeclampsia groups may be associated with the physiological aspects of pregnancy and the increased activity of SOD. The high levels of erythrocytary zinc during pregnancy showed that there was an adequate nutritional status of the mineral, which is consistent with the activity of SOD.

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