Plasma nitric oxide, endothelin-1, arginase and superoxide dismutase in the plasma and placentae from preeclamptic patients

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ABSTRACT

The aim of this study was to determine parameters of NO metabolism in plasma and placenta of preeclamptic (PE) patients. It was conducted a case-control study at São José Hospital, Brazil. Thirty-three PE and 33 normotensive pregnant were included in the study. The diagnosis of PE was established in accordance with the definitions of American College of Obstetricians and Gynecologists. Peripheral venous blood and placenta samples were obtained at postpartum period. Plasma NO levels and SOD activity were significantly lower and endothelin-1 levels and arginase activity were significantly higher in PE women when compared to controls. None of the analyzed parameters were different in the placenta between groups. Our findings suggest that parameters associated with NO metabolism are altered only at the systemic level, but not in placenta of PE patients.

Key words: preeclampsia, superoxide dismutase, arginase activity, endothelin-1 levels, nitrite/nitrate.

INTRODUCTION

Preeclampsia (PE) is a hypertensive and multiple-system disorder that occurs during human pregnancy. It has an unknown etiology, and is defined by an onset of proteinuria, hypertension and edema after 20 weeks of gestation (Wagner 2004). PE affects about 2 to 3% of all pregnancies and it is an important cause of maternal death and the leading cause of intrauterine growth restriction (Redman and Sargent 2005). In PE it is described abnormalities of fetoplacental blood flow, which are characterized by abnormal umbilical blood flow velocity waveforms, indicative of increased placental resistance (Okumura et al. 1999). It is suggested that the placenta have a major role in PE development for several proposed mechanisms. Probably one of the most relevant is that placenta releases factors into the maternal circulation that leads to maternal endothelial dysfunction (Kim 2006). Endothelial dysfunction is considered to underlie PE clinical manifestations, such as maternal hypertension, proteinuria and edema (Wang et al. 2007). The vascular endothelium regulates vascular smooth muscle tone by producing vasoconstrictors

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mediators, such as endothelin-1 and thromboxane; and vasodilators mediators such as prostacyclin and nitric oxide (NO). Endothelial injury induces an imbalance between these substances resulting in vasospasm. One of the proposed mechanisms responsible to endothelial damage is oxidative stress (Hubel et al. 1989).

Compelling evidence supports the understanding that endothelial activation/dysfunction and increased oxidative stress are a central pathophysiological event in the maternal vascular system in PE (Gu et al. 2006). The steady-state levels of NO depend on several factors, including arginine concentration, nitric oxide *synthase* (NOS) activity and superoxide anion concentration (Lowe 2000). Lower levels of SOD activity also impede the effects of NO on blood pressure regulation, by allowing it to react with superoxide anion forming peroxynitrite, thereby increasing oxidative stress (Rumiris et al. 2006). Arginase is a key enzyme responsible to the metabolism of nitrogen since it regulates the levels of L-arginine. Thus, an increased activity of arginase could decrease the production of NO (Ash 2004, Xia et al. 1996). Furthermore, there is limited evidence of the occurrence of these alterations in patients with PE, and if they are systemic or limited to the placenta.

In this context, the present study was designed to measure SOD and arginase activities, nitrite/nitrate (Nox) and endothelin-1 content in plasma and placenta of normal and PE patients.

MATERIAL AND METHODS

PATIENTS

The Ethics Committee of São José Hospital approved study protocol, and informed consent was obtained from all participants. The study population consisted of 33 consecutive PE patients and 33 normotensive pregnant admitted to cesarean delivery at the Obstetrics and Gynecology Department between January 31st and December 31st 2009. The diagnosis of PE was established

in accordance with the definitions of American College of Obstetricians and Gynecologists (ACOG criteria practice bulletin 2002). Women with ruptured membranes, multiple pregnancy, medical complications (including autoimmune disorders, diabetes mellitus), inflammatory conditions (including chorioamnionitis) and cases of chronic hypertension with superimposed PE were excluded. Control patients were matched to PE according to maternal age and gestational age at birth. Peripheral venous blood samples were obtained into heparinized vacutainer tubes, approximately at the same time of placenta collection. The maternal section of the placenta was obtained in the postpartum period and it was immediately washed with saline solution to remove blood cells. All biological specimens were conditioned in liquid nitrogen and then stored at -80°C until assayed.

NITRITE/NITRATE CONCENTRATION

Total concentration of nitrite was determined in blood samples and placenta by a modified Griess reaction method (Verdon et al. 1995). Samples were incubated for 3h at 20°C with glucose-6-phosphate (500 μmol/L), glucose-6-phosphate dehydrogenase (160 U/L), NADPH (1 μmol/L) and nitrate reductase (20 U/L) in phosphate buffer (80 mmol/L, pH 7.5). The Griess reaction was then initiated by addition of sulfanilamide to a final concentration of 0.5% (wt/vol), orthophosphoric acid (1.25%, vol/vol), and *N*-(1 naphthyl) ethylenediamine hydrochloride (0.05%, wt/vol). After a further incubation at 20°C for 10 min, the absorbance of each sample mixture was measured at 540 nm and corrected for opacity by measuring the absorbance at 750 nm. The corrected absorbance was interpolated in a standard curve of absorbance plotted *versus* concentration, in order to find the concentration of nitrite in the sample. Results of the nitrite assay were expressed as nitrite concentration in micromoles per mL.

ARGINASE ACTIVITY

Arginase activity was measured as previously described (Loeb and Stuhlman 1969). Briefly, biological samples were incubated with 50 mM Tris-HCl, 10 mM $MnCl_2$ and the enzyme was activated by heating for 10 min at 56°C. Arginine hydrolysis was initiated by the addition of 0.5 M L-arginine, pH 9.7, at 37°C for 45 min. The reaction was stopped with H_2SO_4 (96%)/H_3PO_4 (85%)/H_2O (1/3/7,v/v/v). The urea concentration was measured at 540 nm after addition of 25 mL α-isonitrosopropiophenone (dissolved in 100% ethanol) followed by heating at 95°C for 30 min. Arginase activity was expressed as µM arginine/L/h (13).

SUPEROXIDE DISMUTASE (SOD) ACTIVITY

The activity of SOD was assayed by the epinephrine method based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the autoxidation of adrenaline at 26°C, as previously described (Bannister 1987).

ENDOTHELIN-1 CONTENT (ET-1)

Concentrations of endothelin-1 were determined by a standard sandwich ELISA, employing comercial available Kits (R&D Systems, Minneapolis, MN) according to manufacturer recommendation.

STATISTICAL ANALYSES

Standard descriptive statistics were calculated to examine baseline characteristics of the study population. Continuous variables with normal distribution were presented as mean ± standard deviation and compared by t-Student test. Continuous variables with a non-normal distribution were reported as median (25%-75% interquartile range) and compared using Mann-Whitney U test. Categorical variables were presented as absolute numbers (frequency percentages) and analyzed by Chi-square test. Correlations were performed by the Pearson test. It was calculated a sample size of at least 25 patients for each group to detect a difference between groups of 20% (predicting a standard deviation of 25%), a sigma of 0.05 and power of 0.80. A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

PATIENTS

Clinical characteristics of included patients were shown in Table I. Arginase activity was significantly higher in the plasma of PE group when compared to the control group ($p<0.01$); but it did not differ significantly in the placenta (Figure 1). This same pattern was observed for ET-1 plasma levels (Figure 2). It was demonstrated that the levels of plasma NO were significantly lower in PE when compared to control patients ($p<0.01$), but there were no significant differences in the placenta (Figure 3). In addition, SOD activity was significantly lower in PE group when compared to controls in the plasma ($p<0.01$), but not in the placenta (Figure 4). Furthermore, there were no significant correlation between plasma and placenta levels for any of the measured parameters (data not shown).

TABLE I
Major clinical characteristics of women with normotensive pregnancy and preeclampsia.

	Preeclampsia (n=33)	Normal gestation (n=33)	p-values
Age (years)	25 ± 6	27 ± 7	0.35
Parity (n)	2.3 ± 0.7	1.9 ± 0.8	0.25
Gestational age at delivery (weeks ± days)	37.6 ± 8.5	36.1 ± 8.4	0.42
Systolic blood pressure (mmHg)	150 ± 12	115 ± 9	0.03
Diastolic blood pressure (mmHg)	98 ± 5	73 ± 4	0.002
Pre-pregnancy BMI	23 ± 6	22 ± 6	0.40
Birth weight (g)	2495 ± 500	2948 ± 530	0.02

BMI, body mass index.
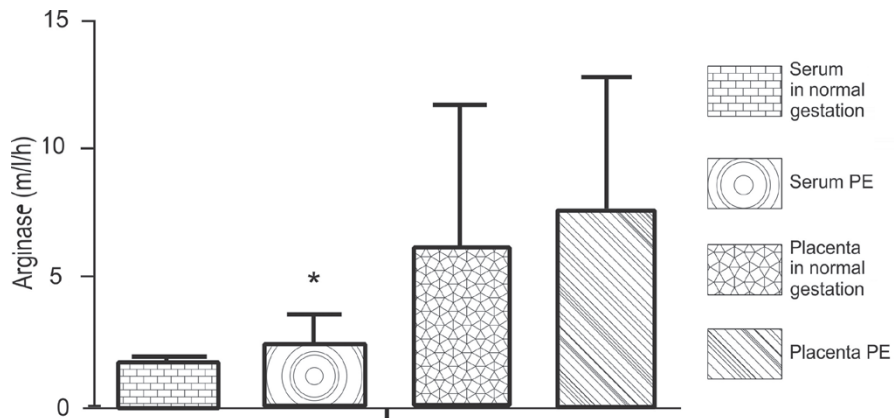
Figure 1 - Arginase activity in serum and placenta in normal gestation and preeclamptic patients. *p<0.01 compared to normal gestation group.
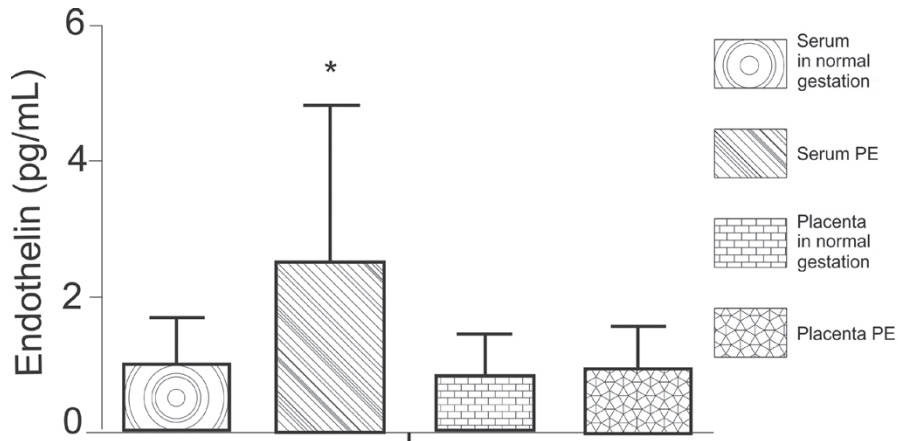

Figure 2 - Endothelin concentration in serum and placenta in normal gestation and preeclamptic patients. *p<0.01 compared to normal gestation group.
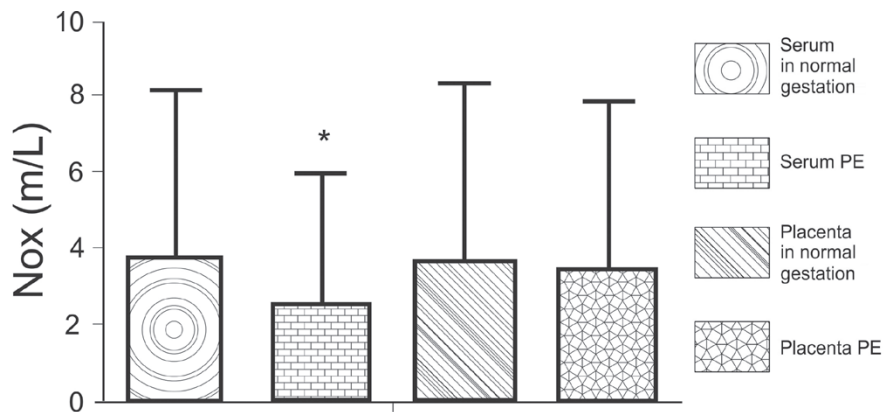

Figure 3 - Concentration NOx in serum and placenta in normal gestation and preeclamptic patients. *p<0.01 compared to normal gestation group.
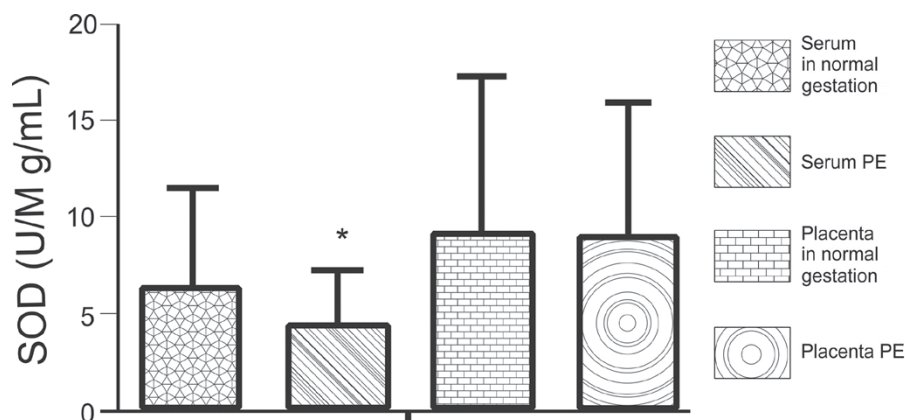
Figure 4 - Superoxide dismutase (SOD) activity in serum and placenta in normal gestation and preeclamptic patients. *$p<0.01$ compared to normal gestation group.

DISCUSSION

We here demonstrated that several different parameters related to NO metabolism were altered in the plasma of PE patients, but differently from what we supposed these same parameters were not altered in the placenta of PE when compared to normal gestation.

PE is a major cause of perinatal and maternal morbidity and mortality. PE is characterized by abnormalities in fetoplacental blood flow. Interestingly, during PE plasma nitrate concentrations, which are an index of NO synthesis, are only slightly reduced (Verdon et al. 1995), as we here demonstrated. This is not uniformly demonstrated in the literature. There are reports of normal and even increased NO levels in PE patients, but a recent meta-analyses suggests that in overall there is a decrease in NO levels in PE patients (Dai et al. 2013). These results must be interpreted with caution since there is significant heterogeneity between included studies.

Thus, it is possible that placenta could better reflects the alterations of NO metabolism, but we could not determine any significant differences in several parameters involved in NO metabolism levels in PE's placenta. The relative concentration of NO in placenta depends on its site and rate of synthesis, its half-life, and its site of action. The interaction of NO with superoxide anion causes its inactivation. Conversely, the activity of NO is prolonged in the presence of SOD, which removes superoxide anions (Loeb and Stuhlman 1969, Bannister 1987). We here demonstrated that NO levels were reduced in plasma of PE women when compared to normal controls, but there was no difference in the placenta as well as SOD and arginase activities were unaltered reinforcing the lack of alterations in the metabolism of NO.

The expression of arginase was increased in placenta of PE patients (Sankaralingam et al. 2010). Our study examined arginase I and observed an increased activity in the plasma, but not placenta, in patients with PE. Arginase exists in two isoforms, arginase I (cytosolic) and arginase II (mitochondrial). The primary function of arginase I and II in the liver and kidney, respectively, are to regulate urea metabolism. In the vasculature, arginase I is expressed both in vascular smooth muscle cells and the endothelium, while arginase II is highly expressed in the endothelium. Thus, it is possible to speculate that arginase is involved in the regulation of the systemic, but not placental, levels of NO during PE.

Clinical evidence from human studies indicates that endothelin plays an important role in mediating pathophysiological changes that occur during PE. Higher ET-1 plasma concentrations were found

in patients with PE (Myatt et al. 1996). Typically, these levels are highest during the latter stage of the disease, suggesting that ET-1 may not be involved in the initiation of PE, but in the progression of disease into a malignant phase (LaMarca et al. 2005). Previous studies have been reported that ET-1 plasma levels can have significant long-term effects on systemic hemodynamics and arterial pressure regulation. Thus, long-term elevations in plasma levels of ET-1 comparable to those measured in women with PE could play a role in mediating the reductions in renal function and elevations of arterial pressure observed in women with PE (Myatt et al. 1996). We observed increase in plasmatic endothelin of patients with PE, but not in placenta, and this suggest that at final stages of the disease systemic endothelial, rather than placental, is relevant to the development of the symptoms.

Some limitations of our study must be pointed-out. As we collected a single time point of biological samples, it is not possible to ascertain a cause-effect relationship between alterations in SOD, arginase and nitrite levels. In addition, due to this limitation is possible that placental NO metabolism is altered at earlier times during PE. Thus, at these later time points we are just able to determine a systemic effect of the presence of PE, but not an earlier pathophysiologic placental alteration.

CONCLUSIONS

Our findings suggest that parameters associated with NO metabolism are altered only at the systemic level, but not in placenta of PE patients. These data suggest that, despite placenta is thought to have a major role in the pathophysiology of PE, some alterations could be restricted to the "systemic compartment", or only at earlier times that we could not access due to limitations of our sample.

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RESUMO

O objetivo deste estudo foi determinar parâmetros de metabolismo de NO no plasma e placenta de pacientes com pré-eclâmpsia (PE). Foi realizado um estudo de caso-controle no Hospital São José, Brasil. Trinta e três PE e 33 gestantes normotensas foram incluídas no estudo. O diagnóstico de PE foi estabelecido em conformidade com as definições do Colégio Americano de Obstetras e Ginecologistas. Amostras de sangue venoso periférico e placenta foram obtidos no período de pós-parto. Os níveis plasmáticos de NO e atividade SOD foram significativamente menores e os níveis de endotelina-1 e da atividade da arginase foram significativamente maiores em mulheres PE quando comparados aos controles. Nenhum dos parâmetros analisados foram diferentes na placenta entre os grupos. Nossos resultados sugerem que os parâmetros associados ao metabolismo do NO estão alterados apenas no nível sistêmico, mas não na placenta de pacientes PE.

Palavras-chave: pré-eclâmpsia, superóxido dismutase, atividade da arginase, níveis de endotelina-1, nitrito/nitrato.

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