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RESISTANCE INDEX OF THE FETAL UMBILICAL ARTERY AND OXIDATIVE STRESS IN PREECLAMPSIA

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OKSIDACIONI STRES I REZISTENTNI INDEKS UMBILIKALNE ARTERIJE U PREEKLAMPSIJI

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ABSTRACT

Preeclampsia is a pregnancy complication with serious consequences for mother and infant. The disorder is diagnosed by hypertension and proteinuria. Several hypotheses invoke oxidative stress as a cellular process contributing to pathologic changes in preeclampsia. Literature data showed that activation and/or dysfunction of the maternal and fetal vascular endothelium may be the consequence of increased oxidative stress. Increased vasoconstriction lead to maternal hypertension and reduced uteroplacental blood flow. Increased vasoconstriction of umbilical artery can be demonstrated using Doppler velocimetry as increased resistance. Our study involved 22 healthy (control) and 20 pregnant women with mild preeclampsia (study group). The aim of this study was to investigate relationship between resistance index of the fetal umbilical artery and concentration of GSH and GSSG (indicators of oxidative stress) in maternal circulation.

Key words: oxidative stress, preeclampsia, fetal umbilical artery

SAŽETAK

Preeklampsija predstavlja za trudnoću specifičan sindrom, koji je praćen povećanim morbiditetom i mortalitetom majke i fetusa. Kliničku sliku karakterišu pojava hipertenzije i značajne proteinurije u drugoj polovini trudnoće. Etiopatogenetska zbivanja tokom preeklampsije još uvek nisu dovoljno razjašnjena, ali nekoliko hipoteza pominje oksidacioni stres kao značajnu komponentu patogeneze preeklampsije. Literarni podaci ukazuju da je za disfunkciju odnosno aktivaciju endotela najverovatnije delom odgovoran i pojačani oksidacioni stres. Hipertenzija i smanjena uteroplacentarna cirkulacija su posledica vazokonstrikcije karakteristične za preeklampsiju. Primenom Doppler ultrazvuka možemo registrovati pojačanu vazokonstrikciju kroz umbilikalnu arteriju. U našoj studiji je bilo ispitano 22 zdrave trudnice (kontrola) i 20 trudnica sa umerenim oblikom preeklampsije. Cilj studije je bio da se ispita odnos između oksidacionog stresa (koncentracije u krvi GSH i GSSG)i rezistentnog indexa (RI) u umbilikalnoj arteriji.

Ključne reči: oksidacioni stres, preeklampsija, umbilikalna arterija

INTRODUCTION

Preeclampsia is human pregnancy-specific syndrome that adversely affects the mother and the fetus, with increased morbidity and mortality. The incidence of preeclampsia is between 4% and 8 % of pregnancies. Preeclampsia is diagnosed by new development of hypertension and significant proteinuria after 20 weeks of gestation. Other manifestations of preeclampsia include reduced perfusion to organs and platelet activation. After delivery, these signs remit [1]. The etiology and pathogenesis of preeclampsia remain poorly understood. Increasing evidence indicates that activation and/or dysfunction of the maternal and fetal vascular endothelium may be the consequence of increased oxidative stress. Increased vasoconstriction lead to maternal hypertension and reduced uteroplacental blood flow [2-4].

Oxidative stress is disturbance of the redox-balance, caused by increased amounts of oxidants such as reactive oxygen species – ROS (anion radical (O_2.-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH.) or a deficiency of antioxidants [5]. Under physiological conditions, overproduced ROS are neutralized by the activity of antioxidative defense system (AOS), which consists of enzymatic and non-enzymatic components [5].

Gluthatione (GSH) is non-enzymatic component of AOS and plays a major role in defenses against oxidative and nitrosative stress. GSH reacts with hydroxyl radical, the cytotoxic Fenton reaction product, and with N_2O_3 and peroxynitrite, cytotoxic products formed by the reaction of nitric oxide (NO) with O_2 and superoxide, respectively. In addition, GSH acts as a co-substrate of

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GPx in the elimination of both H_2O_2 and other organic peroxides. Each of these reactions leads directly or indirectly to the formation of glutathione disulfide (GSSG) [6]. Upon exposure to oxidants, GSH forms GSSG, thus changing the plasma concentration of GSH and GSSG, which reflects oxidative stress conditions [7]. Considering key role of placenta in initiation of oxidative stress in maternal circulation, in this study we investigated relationship between RiAU (resistance index of the fetal umbilical artery) and concentration of GSH and GSSG (indicator of oxidative stress) in maternal circulation.

MATERIALS AND METHODS

Chemicals

All reagents used in this study were of analytical grade and were obtained from Sigma Chemical Company, St. Louis, USA and Merck, Darmstadt, Germany.

Patients

Ethics Committee of Clinical Centre Kragujevac approved the study protocol and all patients gave informed consent. Our study involved 22 healthy pregnant women (control group) and 20 women with mild preeclampsia (study group), attending the Obstetrics and Gynaecology Department. Preeclampsia was defined by standard criteria in accordance with the guidelines of the International Society for the Study of Hypertension in Pregnancy. Control and preeclamptic women were non-smokers and free from other disease.

Preeclampsia is one of indications for use of Doppler ultrasound during pregnancy. The degree of resistance in a vascular segment can be ascertained. This information is used to determine the condition of the fetus, especially in the third trimester. In preeclampsia, there is reduction in placental perfusion, which could be detected by Doppler measurements as increased resistance to blood flow through feto-placental vessels [8]. The Doppler ultrasound investigation was performed on ALOKA Pro Sound 3500 SSD. We measured index of resistance to blood flow through umbilical artery.

Blood sampling and analytical methods

Blood samples were taken by venepunction from pregnant women in third trimester of pregnancy. The blood was collected in sterile tubes with preservative-free sterile heparin (40 U/ml) and centrifuged 10 min at 5000 rpm. After blood centrifugation plasma of healthy and preeclamptic women were used for extraction and determination of the next parameters: concentrations of reduced glutathione (GSH) and oxidized glutathione (GSSG). Extract of GSH and GSSG were stored at –80°C until further biochemical analysis. Levels of GSH was determined on the basis of GSH oxidation with 5,5-dithio-bis-6,2-nitrobenzoic acid [9]. Concentration of GSSG was enzymatically determined by glutathione reductase [10] after inhibition of GSH oxidation by N-ethylmaleimide. Concentration of GSH and GSSG were expressed in nmol/ml of plasma.

Statistical analysis

The data are expressed as mean ± S.E.M. The data were analyzed by SPSS (version 10.0) for Windows. Paired samples t-test was used for comparison of the data. Correlation coefficient (r) was determined by Pearson correlation. For comparisons and correlation $p < 0.05$ was considered as significant.

RESULTS

Clinical data of pregnant women are summarized in Table 1. Maternal age and parity were not significantly different between the groups. As anticipated from the defined criteria, MAP (mean arterial pressure) and proteinuria were significantly higher in the preeclamptic group than in the control group. Gestational ages at delivery and infant birth weights were significantly lower in the preeclamptic group than in the control group. Apgar score was not different between the groups.

Table 1. Clinical characteristics of healthy (control) and preeclamptic pregnant women

	Control	Preeclampsia
Characteristic		
	(n = 22)	(n = 20)
Maternal age (years)	26.9 ±4.5	28.1 ±5.5
Parity	1.4 ±0.2	1.5 ±0.2
Gestational age at delivery (w)	40.36 ±0.53	38.52 ±0.90*
MAP (mean arterial pressure) (mmHg)	91.08 ±1.99	109.54 ±1.66*
Proteinuria	0.00 ±0.00	0.54 ±0.035*
Infant birth weight (g)	3736 ±0.08	3259 ±0.11*
Apgar score	8.78 ±0.14	8.82 ±0.16

Values are means ± SEM. *$p < 0.05$ preeclampsia versus control

The resistance index of the fetal umbilical artery is presented in Fig. 1. Results of this study showed that RiAU was significantly higher in preeclampsia in comparison to healthy pregnant women. Concentrations of GSH and GSSG are present in Fig. 2 and Fig. 3. Our results showed significantly higher concentrations of GSH and GSSG in plasma of preeclamptic women in comparison to healthy pregnant women. The results of this study show that RiAU was significantly negatively correlated with concentration of GSH in plasma of preeclamptic women ($r = – 0.847$, $p < 0.0001$). Negatively correlation between RiAU and concentration of GSSG was not significant in preeclampsia.

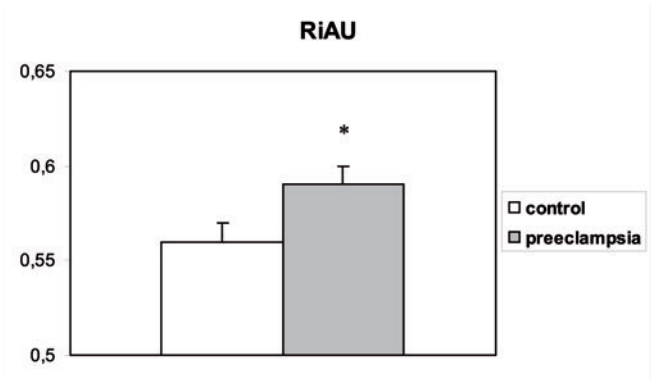


Figure 1. RiAU in women with preeclampsia and in healthy women. Values represent means ± SEM.
*p < 0.05, preeclampsia versus control.

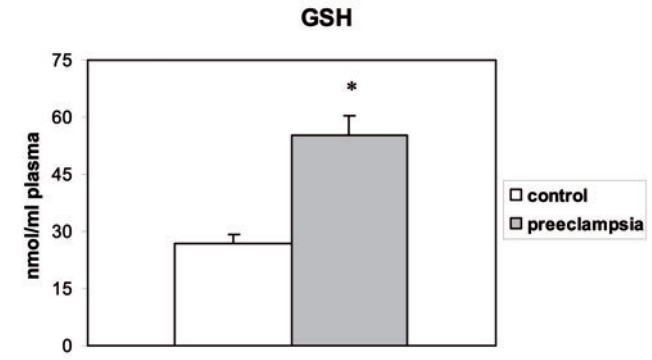


Figure 2. The concentrations of GSH in women with preeclampsia and in healthy women.
Values represent means ± SEM. *p < 0.05, preeclampsia versus control.

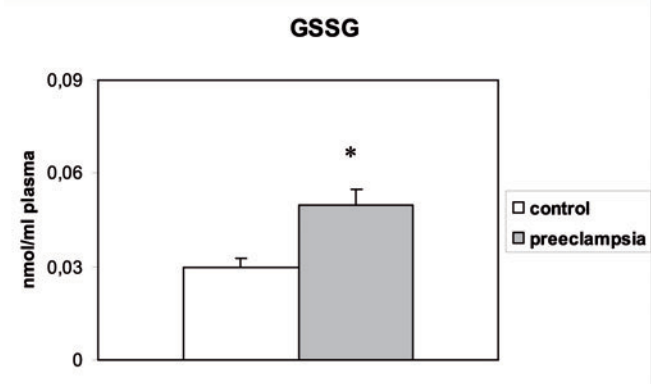


Figure 3. The concentrations of GSSG in women with preeclampsia and in healthy women. Values represent means ± SEM. *p < 0.05, preeclampsia versus control.

DISCUSSION

Oxidative stress is the link between the poor placental perfusion and the impaired maternal endothelial function occurring in preeclampsia [1, 4]. Placental ischemia may promote the release of a variety of biologically active factors, including cytokines such as tumor necrosis factor-alpha (TNF-α), which induce neutrophils to discharge reactive oxygen species. Threshold increases in the plasma levels of placental factors may lead to endothelial cell dysfunction [11-14]. The release of factors from hypoxic placenta may also increase the release of or the vascular reactivity to endothelium-derived contracting factors such as endothelin (ET-1), thromboxane, and ANG II [15].

Production of free radical is relatively low in normal conditions due to active antioxidative defense systems, including GSH. Reduced glutathione is an endogenous scavenger of free radicals and peroxides because it maintains the redox potential and is very protective against oxidative stress [6, 7]. The changes in plasma concentration of GSH suggest increased oxidative stress [4, 5]. In this study, we showed higher concentration of GSH in plasma of preeclamptic women in comparison to healthy pregnant women. Literature data show that TNF-α increases hepatocellular GSH levels, mediated by transcriptional regulation of the GCS-HS gene, which attenuates the generation of hydrogen peroxide and lipid peroxidation [16]. Scalera et al. [17] showed that higher concentration of ET-1 also increases the intracellular concentration of GSH. Glutathione efflux from liver by TNF-α resulted in significant elevation of the plasma GSH concentration [18]. Increase of plasma GSH represent an additional protective mechanism to control the consequences of oxidative stress induced by inflammatory cytokines in preeclampsia.

While deactivating reactive oxygen species, the reduced form of glutathione is oxidized, thereby changing the ratio of free and oxidized glutathione [5]. The results of this work show that concentration of GSSG was significantly higher in plasma of women with preeclampsia, while ratio GSH/GSSG is not different between the study groups. Because preeclampsia is characterized by oxidative stress, we anticipated that ratio of GSH and GSSG would be lower in women with preeclampsia. No change in GSH/GSSG ratio may be result of rapidly reduced GSSG by glutathione reductase, thereby consuming nicotinamide adenine dinucleotide phosphate. In addition, when increasing oxidized glutathione, some of the GSSG undergo renal degradation by gammaglutamyl transpeptidase, resulting in an irreversible loss of GSSG [19]. Because of one or both of these mechanisms, the ratio of GSH and GSSG remained unchanged.

Okatani et al. [20] showed that H_2O_2 is potent vascular tension in human umbilical arteries. General vasoconstriction in preeclampsia, mediated by oxidative stress, results with high flow resistance in the capillaries of the terminal villi, and with a low end-diastolic velocity in the umbilical artery and consequent hypoxia [2, 4]. Because of the prolonged fetal hypoxia, circulatory adaptation occurs in the form of cerebral vasodilatation, resulting in the redistribution of the cardiac output to provide an adequate oxygen supply to the brain. These changes, which help fetus to adapt to a hostile environment, may correlate with fetal neonatal health [21].

Because GSH acts as a co-substrate of GPx in the elimination of H_2O_2, we investigated correlation between RiAU and concentration of maternal GSH in plasma. On the basis of our results, RiAU increases and is negatively correlated with GSH in preeclampsia. This suggests that increased concentration of GSH in maternal plasma is protective mechanism against oxidative injury in human umbilical arteries.

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