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THIOBARBITURIC ACID REACTIVE SUBSTANCES AS MARKER OF OXIDATIVE STRESS IN PREGNANCIES WITH PRE-ECLAMPSIA

TIOBARBITURNA KISELINA KAO MARKER OKSIDATIVNOG STRESA U TRUDNOĆAMA KOMPLIKOVANIH PREEKLAMPSIJOM

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Summary - Pre-eclampsia is characterized by increased lipid peroxidation and diminished antioxidant capacity. The aim of the study was to establish concentration of thiobarbituric acid reactive substances as a marker of lipid peroxidation in normal pregnancies and in pregnancies complicated with pre-eclampsia, and to estimate the possibility of using thiobarbituric acid reactive substances as a screening method for development of pre-eclampsia. The study was conducted at the Department of Obstetrics and Gynaecology, Clinical Centre of Vojvodina. The study included 57 singleton pregnancies, gestation ≥24 weeks, of which 29 were healthy pregnancies and 28 were with pre-eclampsia, defined as systolic arterial pressure of ≥90 mmHg, diastolic of ≥145 mmHg, and 24h proteinuria of ≥300mg. Thiobarbituric acid reactive substances concentrations evaluated by malondialdehyde equivalent standards (OxiSelect™ TBARS Assay Kit (malondialdehyde Quantitation), Cell Biolabs' OxiSelect™) showed that oxidative stress was more evident in the group with pre-eclampsia, though not statistically significant (p= 0.107). There was no correlation of thiobarbituric acid reactive substance levels with gestation in either group. The differences between the level of thiobarbituric acid reactive substance concentrations in pre-eclampsia and healthy pregnancies indicate the possibility of using thiobarbituric acid reactive substances as a screening tool for the development of pre-eclampsia. Further studies with larger numbers of patients are needed in order to come to final conclusions.

Key words: Oxidative Stress; Pregnancy Complications; Thiobarbituric Acid Reactive Substances + diagnostic use; Pre-Eclampsia; Lipid Peroxidation; Malondialdehyde; Female; Hypertension; Proteinuria

Introduction

Pathological pregnancies resulting from placental changes can be found almost only in humans. These disorders, seen in about 30% of human pregnancies, include primarily spontaneous abortion and pre-eclampsia (PEE). In other mammals, the incidence of both of these disorders is very low [1], with PEE appearing only in one species of monkeys [2]. In humans, the modern way of life, which includes postponement of pregnancy and hyper caloric diet, may be the cause of increase in incidence of PEE [3]. PEE is a result of defective early trophoblast invasion – although trophoblast invasion is appropriate for concept implantation, it is inadequate for a full conversion of spiral arteries into low resistance channels [4]. A result of inadequate conversion of spiral arteries is retention of smooth muscles in their walls, so that vasoreactivity of vascular pool persists in about 30–50%, which may lead not only to decreased perfusion of intervillous spaces, but also, more importantly, to intermittent perfusion. Since the placenta and fetus continuously extract oxygen, the result may be transitory hypoxia [5] with consequential chronic damage.

Increased oxidative stress in placenta of women with PEE is well documented [6-8]. The imbalance of oxidative/antioxidative relation affects more sensitive tissues, which leads to lipid peroxidation. In cases of increased oxidative stress, lipid perodixation leads to damage of unsaturated fatty acids in cell membranes [9]. Lipid peroxidation is directly involved in the process of maternal endothelial dysfunction by increase of thromboxane A2 production. The level of lipid peroxidation is increased in pregnancy above the non-pregnant levels even in normal pregnancies, which indicates certain degree of physiological oxidative stress [10,11]. Free fatty acids are particularly sensitive to oxidation and increase 15 to 20 weeks prior to PEE [12]. Changes in concentrations of some markers of oxidative stress precede the development of clinical symptoms, which indicates the primary phenomenon of chronic oxidative

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Abbreviations

TBARS	– thiobarbituric acid reactive substances
TAS	– systolic arterial pressure
TAD	– diastolic arterial pressure
MDA	– malondialdehyde
PEE	– pre-eclampsia
oxLDL	– oxidative lypoprotein of the low density

stress [13]. Unlike in abortion, where the degeneration of placental tissue is fast and generalized, the placental degeneration is progressive in PEE and it may be compensated for a certain period of time, depending on the degree of initial placental defect and basal antioxidative capacity of placenta.

Thiobarbituric acid reactive substances (TBARS) are biochemical parameters of oxi-redox homeostasis, pro-oxidative marker and well defined method for screening and monitoring of lipid peroxidation.

The aim of the study was to establish the concentrations of markers of oxidative stress in healthy pregnant women and pregnant women with symptoms of PEE.

Material and Methods

The study was conducted at the Department of Obstetrics and Gynaecology, Clinical Centre of Vojvodina, in the period April/December 2010, after getting the approval of Ethics Committee of Clinical Center of Vojvodina. Sixty patients were included in the study: 30 pregnant women with PEE formed the study group and 30 healthy pregnant women, with uncomplicated pregnancies were in the control group. All patients signed the informed consent to participate in the study.

The inclusion criteria for the study group were: primipara, singleton pregnancy, gestation of 24 weeks and more, systolic arterial pressure (TAS) of 90 mmHg and higher, diastolic arterial pressure (TAD) of 145 mmHg and higher, 24h proteinuria above 300 mg, lack of other complications except symptoms of pre-eclampsia. The inclusion criteria for the control group of healthy pregnant women were: primipara, singleton pregnancy, gestation of 24 weeks and more, no complications of pregnancy, no chronic diseases before and during the ongoing pregnancy. The exclusion criteria in the study group were the development of other complications except PEE symptoms, and in the control group, the development of any complications of pregnancy (high blood pressure, proteinuria, pathological values of carbohydrate metabolism, acute infections, bleeding, preterm rupture of membranes, preterm delivery, etc).

Two ml of plasma was taken from every patient, and frozen at -20 degrees of Celsius. TBARS levels were determined by the equivalent malondialdehyde (MDA) standards, according to the recommendations of test manufacturer (OxiSelect™ TBARS Assay Kit (MDA Quantitation), and the result was obtained by spectrophotometry.

Results

During the study, the results from 57 pregnant women were analyzed and divided into two groups - PEE group (n=28 women) and the group of healthy pregnant women, the same gestations as the women in the study group (the control group, n=29). The gestations in both groups were adequately paired **(Table 1)**.

Table 1. Comparison of gestations
Tabel 1. *Komparacija gestacija*

		Groups/*Grupe*		Total/*Ukupno*
		PEE	No PEE/*Nema PEE*	
Gestation/*Gestacija*	24	2	1	3
	25	1	1	2
	27	2	2	4
	28	3	3	6
	29	1	1	2
	30	1	2	3
	31	1	1	2
	32	4	4	8
	33	2	2	4
	34	4	4	8
	35	1	1	2
	36	5	6	11
	37	1	1	2
Total/*Ukupno*		28	29	57

The gestational age was approximately the same in the study subjects and the controls. The average gestation in the PEE group was 31.54 gestational weeks (min 24, max 37, SD=3.92), and in the control group, it was 31.9 gestational weeks (min 24, max 37, SD=3.66). The difference was not statistically significant (p=0.72) **(Table 2)**.

Table 2. Gestational age
Tabel 2. *Gestacijska starost*

Parameter/*Parametar*		N	Mean/*Prosek*	SD	Min	Max	p
Gestation	PEE	28	31.54	3.92	24	37	0.721
Gestacija	No PEE/*Nema PEE*	29	31.90	3.66	24	37	
	Total/*Ukupno*	57	31.72	3.76	24	37	

The pregnant women were of approximately the same age in the study and in the control group, it being 30.43 years (SD 6.24), and 27.9 years (SD 5.7), respectively; the difference was not statistically important (p=0.116).

The difference of the TBARS in the two groups was established by a Mann Whitey non-parametric test because of the large amount of deviation found between the two groups. The average value of TBARS was 36 μM (min 9.6, max 112, SD 25) and 24.8 μM (min 5.71, max 56.3, SD 16.4) in the group with pregnant women with PEE and in the group of women without PEE, respectively. The difference between the concentrations of TBARS in the two groups was not statistically significant (p=0.107) **(Table 3)**.

Table 3. TBARS Concentrations
Tabela 3. *TBARS koncentracije*

Parameter/*Parametar*		N	Mean/*Prosek*	SD	Min	Max	P
TBARS	With PEE/*Ima PEE*	28	36,0	25.0	9.60	112	0.107
	No PEE/*Nema PEE*	29	24.8	16.4	5.71	56.3	
	Total/*Ukupno*	57	30.3	21.6	5.71	112	

Discussion

Pregnancy-induced hypertension, pre-eclampsia and eclampsia are parts of hypertensive syndrome which is a life-threatening condition both for the mother and fetus [14]. Pregnancy itself is a state of increased oxidative stress, and the increase of the oxidative damage is considered important for the development of pregnancy complications such as PEE. TBARS values above 20 μM indicate oxidative stress, so that, based on the TBARS levels of the PEE group and the control group in our study, it is shown that oxidative stress is found in pregnancy, and that it is increased in the PEE group compared to the healthy controls, although not statistically significantly (p=0.107). Raijkers et al [15] showed that the levels of oxidative lypoprotein of low density (oxLDL) were lower in women with PEE than in normotensive pregnant women of the same gestational age (181±12 mmHg vs. 219±14 mmHg; p=0.027), while there was no difference in the concentrations of TBARS. In this study, TBARS values were higher in women with PEE when compared with the TBARS values in pregnant women at term (3.8±0.6 vs. 1.5±0.2; p= 0.01). In pregnant normotensive women, however, TBARS values were lower at term compared to the values in women with uncomplicated pregnancies in the early third trimester (p<0.0001), which indicates that plasma concentrations of TBARS decrease during the third trimester. The women with PEE had also lower values of plasma concentration oxLDL compared with the controls of the same gestations, which indicates that oxLDL may be a marker for PEE. In our study, there was no correlation of TBARS values with the gestation either in the PEE or in the healthy pregnant women group.

By comparing the concentration activity of calcium-activated adenosine triphosphatase (Ca-ATPase) and TBARS of plasmatic membranes from myometriums and trophoblast of normotensive women and women with PEE it was found that the activity of Ca-ATP myometrial and placental trophoblasts in women with PEE was about 50% lower than in normotenive women, while the TBARS levels were lower. This can be the result of high cytosol concentrations of calcium in the smooth cells of blood vessels in women with PEE and it can be explained partly by the development of high pressure in these women [16].

Plasma concentrations of TBARS as a product of lipid peroxidation, protein carbonyl, the product of protein oxidation and a tumor necrosis factor α (TNF-α) and IL-6, which assesses the inflammation, are higher in the women with PEE than in normotensive women at around 20 gestational weeks [17].

In the meta-analysis of the studies done until 2008 in which the Cochrane database was used, it was shown that there was an increase of malondialdehyde, the product of lipid perodixation, in the women with PEE when compared to the controls (95% CI: 0.76, 1.66). Compared with the controls, the total standardized difference in the values of TBARS in serum was by 1.62 nmol/mL higher in women with PEE (95% CI: 0.27, 2.96), while the serum concentrations of vitamin E and vitamin C were lower in PEE than in healthy women (95% CI: -1.77, -0.48; 95%CI: -1.03, -0.02). The overall standardized difference for superoxide dismutase, the important antioxidant enzyme, was -2.37 (95% CI: -4.76, 0.03), which is marginally statistically significant decrease in PEE compared to the controls. This study has shown that in PEE there are not only higher concentrations of markers of oxidative stress, but also lower concentrations of antioxidative substances [18].

Conclusion

Although the results of our study do not offer final conclusions about the mechanism of the role of oxidative stress in the pathogenesis of pre-eclampsia, they may have a role in further research in this field, with the larger numbers of patients since they show the differences in the level of enzyme activities in oxidative stress in pregnant women with pre-eclampsia and open new opportunities in defining markers for the screening of population of pregnant women for the development of pre-eclampsia.

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Sažetak

Uvod

Preeklampsija je stanje povećane lipidne peroksidacije i smanjenog antioksidantnog kapaciteta. Cilj ovog istraživanja bio je da se utvrdi kretanje nivoa tiobarbiturne kiseline (thiobarbituric acid reactive substances) kao markera lipidne peroksidacije u trudnoćama sa preeklampsijom i u zdravim trudnoćama, kao i da se sagleda mogućnost korišćenja ovog markera u skriningu populacije trudnica za razvoj preeklampsije.

Materijal i metode

Ispitivanje je sprovedeno na Klinici za ginekologiju i akušerstvo Kliničkog centra Vojvodine, tokom 2010. godine. Ukupno je uključeno 57 jednoplodnih trudnoća gestacije 24 i više nedelja, 29 bez komplikacija trudnoće, 28 sa simptomima preeklampsije, koja je difinisana kao stanje sa sistolnim arterijskim pritiskom od >90 mmHg, dijastolnim arterijskim pritiskom od >145 mmHg, te 24-časovnom proteinurijom od >300 mg. Nivo tiobarbiturne kiseline određivan je malondialdehidom ekvivalentnim standardima, shodno preporuci proizvođača (OxiSelect™ TBARS Assay Kit (MDA Quantitation), Cell Biolabs' OxiSelect™).

Rezultati

Koncentracije tiobarbiturne kiseline bile su više kod trudnica sa preeklampsijom nego kod zdravih trudnica, mada ne statistički značajno (p=0,107). Nije bilo korelacije koncentracija tiobarbiturne kiseline sa gestacijom ni u jednoj grupi.

Zaključak

Razlike u nivoima koncentracija tiobarbiturne kiseline između trudnica sa preeklampsijom i zdravih trudnica ukazuju na mogućnost korišćenja tiobarbiturne kiseline kao markera u skriningu za razvoj preeklampsija. Potrebna su dalja ispitivanja, sa većim brojem pacijenata radi donošenja konačnih zaključaka.

Ključne reči: *Oksidativni stres; Komplikacije u trudnoći; Tiobarbiturna kiselina + dijagnostička upotreba; Preeklampsija; Lipidna peroksidacija; Malonilaldehid; Žensko; Hipertenzija; Proteinurija*

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