



Disturbance of oxidative balance in the first trimester of spontaneous abortions

Poremećaj redoks ravnoteže kod žena sa spontanim pobačajima u prvom trimestru trudnoće

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Abstract

Background/Aim. Pregnancy is defined as a condition of increased oxidative stress. The aim of this research was to determine the intensity of pro-oxidative processes and the content of GSH, as well as antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and the total antioxidative status (TAS) in patients with spontaneous abortions. **Methods.** A total of 120 patients were involved in the research (70 spontaneous abortions and 50 healthy pregnancies). The patients were divided into groups: 35 patients with incomplete and complete spontaneous abortion (group S), 35 patients with missed abortion (group M) and a control group of 50 healthy pregnancies (group N), all of them being in the first trimester of pregnancy. The intensity of lipid peroxidation (LPx) was determined with a modified thiobarbituric acid method. The GSH content in erythrocytes was determined by the method based on the amount of non-protein sulphydryl residues using the Ellman's reagents. The following antioxidative parameters in the blood were measured: SOD – by the method with xanthine oxidase-using commercial RANSOD sets; CAT – by the method of Aebi (the enzyme activity was measured by monitoring the decomposition of H₂O₂ at 240 nm); GSH-Px was determined using hydrogen peroxide as a substrate. The TAS was determined using the ferric reducing

antioxidant potential (FRAP) method. **Results.** The highest average value of LPx was recorded in the spontaneous abortion group (48.03 pmol/mg Hgb), and the lowest value was recorded in the control group (26.06 pmol/mg Hgb). A statistically significant positive correlation between LPx and CAT in the group of patients with missed abortion was also noted ($p < 0.05$, $r = 0.37$). There was a statistically highly significant difference ($p < 0.001$) in SOD and in CAT activities between the examined patients (groups S and N) and the control group (Student's *t*-test and ANOVA). The highest average value of TAS was recorded in the group S (710.39 μmol/L), while the value in the group M was 277.66 μmol/L. The average value of TAS in the control group was 452.12 μmol/L. Student's *t*-test showed a statistically highly significant difference in the values of TAS between the examined patients (groups S and M) and the control group. **Conclusion.** Determination of the value of pro-oxidative and antioxidative parameters in patients with spontaneous abortion can be the indicator of condition of fetoplacental unit and these analyses can be included in the protocol of the routine perinatal diagnostics.

Key words:

oxidative stress; abortion, spontaneous; lipid peroxidation; superoxide dismutase; catalase; glutathione; glutathione peroxidase; gravidity.

Apstrakt

Uvod/Cilj. Trudnoća se definiše kao stanje povišenog oksidativnog stresa. Cilj rada bio je da se odredi intenzitet prooksidativnih procesa i sadržaja glutationa (GSH), kao i antioksidativnih enzima: superoksid dismutaze (SOD), katalaze (CAT), glutation peroksidaze (GSH-Px) i totalnog antioksidativnog statusa (TAS) kod žena sa spontanim pobačajem. **Metode.** U istraživanje je bilo uključeno 120 žena (70 sa spontanim pobačajem i 50 zdravih trudnica). Žene su bile podeljene u grupe: 35 žena sa nekompletnim i kompletnim spontanim pobačajem (grupa S), 35 žena sa

missed abortusom (grupa M) i kontrolna grupa od 50 zdravih trudnica (grupa N) u prvom trimestru trudnoće. Intenzitet lipidne peroksidacije (LPx) određivan je modifikovanom metodom sa tiobarbiturnom kiselinom. Sadržaj GSH u eritrocitima određivan je na osnovu količine neproteinskih sulfhidrilnih ostataka pomoću Ellman-ovog reagensa. Od antioksidativnih parametara u krvi vršeno je određivanje: SOD – metodom sa ksantin oksidazom, komercijalnim setovima RANSOD; CAT – Aebi-jevom metodom (aktivnost enzima merila se praćenjem razlaganja H₂O₂ na 240 nm); aktivnost GSH-Px određivana je pomoću vodonik-peroksida kao supstrata. TAS je određivan sa *ferric reducing*

antioxidant potential (FRAP) metodom. **Resultati.** Najveća prosečna vrednost LPx zabeležena u grupi žena sa spontanim pobačajem (grupa S), 48,03 pmoL/mg Hgb, a najniža vrednost zabeležena je u kontrolnoj grupi (26,06 pmoL/ mg Hgb). Takođe, zapaženo je postojanje pozitivne korelacije između LPx i CAT ($p < 0,05$, $r = 0,37$) u grupi žena sa *missed* abortusom (grupa M). Postoji statistički visokoznačajna razlika ($p < 0,001$) u vrednostima SOD i CAT između žena u grupama S i M i kontrolne grupe (t -test i ANOVA). Najviša prosečna vrednost TAS zabeležena je u grupi S (710,39 $\mu\text{mol/L}$), dok je vrednost u grupi M bila 277,66 $\mu\text{mol/L}$. Vrednosti TAS u kontrolnoj grupi zdravih trudnica iznosila je 452,12 $\mu\text{mol/L}$. Vrednosti t -

testa i ANOVA pokazuju da postoji statistički visokoznačajna razlika u vrednostima TAS između žena sa abortusom (grupe S i M) i kontrolne grupe. **Zaključak.** Određivanje vrednosti pro- i antioksidativnih parametara kod žena sa spontanim pobačajem može biti pokazatelj stanja fetoplacentarne jedinice i ove analize u budućnosti mogu biti uvrštene u protokol rutinske prenatalne dijagnostike.

Ključne reči:

stres, oksidativni; abortus, spontani; lipidi, peroksidacija; peroksid dismutaza; katalaza; glutation; glutation peroksidaza; trudnoća.

Introduction

Human reproduction is nowadays no longer considered to be a highly efficient biological process. Before the end of the first trimester of pregnancy, 30–50% of conceptions result in spontaneous abortions. A greater number of conceptions are lost during implantation, while 15–20% of clinically proven pregnancies result in spontaneous abortions¹. Recurrent, spontaneous abortions occur in 0.5–3% of women in the reproductive period of their life, and 50–60% are idiopathic².

Pregnancy is defined as a condition of increased oxidative stress. In normal pregnancy, during the earliest stages, the development of the embryo occurs in a low O₂ level environment. This physiological hypoxia in the early gestational sac protects the fetus from teratogenic effects of oxygen free radicals. Once the embryogenesis has been complete, the maternal intervillous circulation is completely established, and the intraplacental concentration of O₂ rises³.

In spontaneous abortions, the development of placental decidual basis is highly disrupted. Some studies show that the systemic and placental oxidative stress have a role in pathophysiological mechanism of spontaneous and recurrent miscarriage occurrence³. The oxidants induce damage to the endothelium, damage to the placental vascularisation, immune malfunctions, and have a major role in the pathophysiology of idiopathic recurrent miscarriages. In a physiological pregnancy the antioxidative mechanisms compensate for the intensified pro-oxidative processes. The spontaneous abortion is coupled with a heavy disruption of the pro-oxidative-antioxidative homeostasis, *ie* the disruption of the antioxidative defense system. According to the literary data lipid peroxidation and antioxidative protection components are significantly changed during pregnancy as compared to the condition without pregnancy^{4,5}. Most of the authors have established an increase in lipid peroxidation during pregnancy⁶. The study of Burton and Jauniaux⁷ indicates an increase in lipid peroxidation intensity in the placenta as well as in the uterus of the pregnant woman.

Some controversial data have been found in the literature concerning the changes of certain antioxidative parameters in pregnancy⁸. The review of all previous research literature data has confirmed that the antioxidative response in pregnancy of healthy women is present to a degree that pro-

vides protection from the increased oxidative risk and is a part of physiology of the pregnancy itself^{9–11}.

The attempts to understand the etiopathogenesis of spontaneous abortions at the molecular level have been under way throughout the world for decades and this research has led to partial explanations of this problem, yet the cause behind most spontaneous abortions remains unknown^{12,13}. By diagnosing the values of antioxidative enzymes, in our study cases of spontaneous abortions, we can acquire additional parameters for diagnosing the idiopathic and recurrent spontaneous abortions.

The aim of this work was to determine the intensity of pro-oxidative processes: the values of lipid peroxidation (LPx) and the contents of the reduced glutathione (GSH), as well as the antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and the total antioxidative status (TAS) in patients with spontaneous abortions.

Methods

This randomized, prospective, comparative study was conducted on a sample of pregnant women with spontaneous abortions at the Clinical Center of Vojvodina, Department of Obstetrics and Gynecology, in Novi Sad during the 2011–2012 period.

Measuring of lipid peroxidation and antioxidative enzymes in the samples was carried out in the laboratory of the Clinical Center of Vojvodina, Department of Obstetrics and Gynecology in Novi Sad, and the Faculty of Medicine, Department of Pharmacy, University of Novi Sad. Measuring of the glutathione contents and the total antioxidative status was carried out at the Faculty of Medicine, Institute of Pharmacology, Toxicology and Clinical Pharmacology, University of Novi Sad. The prospective research included 120 patients, 70 of which had spontaneous abortions in the first trimester of pregnancy and 50 of which had healthy pregnancies of the same age of gestation. The examinees were divided into two groups: the first groups comprised of 35 patients with incomplete and complete spontaneous abortions (the group S) and the second group comprised of 35 patients with missed abortions (the group M), as well as the control group of 50 healthy pregnancies (group N), all of them being in the first trimester of pregnancy.

The samples of erythrocyte and serum hemolysates from women with spontaneous abortions and healthy pregnancies in the first trimester of pregnancy were collected, prepared and stored. By puncturing the cubital vein, 5 mL of blood was collected into the vacutainer lined with ethylenediaminetetra acetic acid (EDTA). The preparation of hemolysates was performed, the serum was separated and frozen. Within the time intervals of 3 months, the collected samples were thawed and smaller series of all of the examined analyses were performed. All of the samples, including the control samples, were analyzed at the same time.

The intensity of LPx was determined by a modified method using thiobarbituric acid¹⁴. The GSH contents in erythrocytes was determined by method based on the amount of non-protein sulfhydryl remnants using the Ellman's reagent, by colorimetric analysis according to Kapetanović and Mieyal¹⁵. The following antioxidative parameters in the blood were measured: SOD, using the xanthine oxidase method – the commercial RANSOD kits¹⁶; CAT using the Aebi's method¹⁷, (enzyme activity was measured by monitoring the decomposition of H₂O₂ at 240 nm); the GSH-Px activity was measured using the hydrogen-peroxide as a substrate, by a modified method using cumene hydroperoxide¹⁸. The total antioxidative status (TAS) was measured using the ferric reducing antioxidant potential (FRAP) method according to Benzie and Strain¹⁹.

This research was approved by the Ethics Committee of the Clinical Center of Vojvodina and Faculty of Medicine, University of Novi Sad. All the patients signed a consent form.

The collected data were presented in the form of median values and relative numbers (the measure of variability). The statistical significance of noted differences was tested using the parametric (Student's *t*-test) and ANOVA-MANOVA test. Statistical data processing was performed using the programs Excel and PSS.

Results

All of the patients participating in our research were uniform according to their age, body weight (BW), body height (BH) and the body mass index (BMI) (Table 1).

The median values of LPx in the examined patients (groups S and M) and the control group (N) were within the range of 26.06–48.03 pmoL/mg Hgb (Table 2). The minimum value of LPx in the patients of all the three examined groups was 8 pmoL/mg Hgb, and the maximum value was 89 pmoL/mg Hgb. Analyzing the acquired values from the *t*-test, we found a statistically significant difference ($p < 0.001$) in the LPx values between the patients in the examined groups and the control group (N : M; N : S). The analysis of the ANOVA-test results proved the existence of a statistically significant difference in LPx values between the groups M : N; S : N.

The median values of GSH in the examined patients (groups S, M) and the control group (N) were within the range of 2.68–3.10 μ mol/mL Er (Table 3). The minimum value of GSH in the patients of all the three examined groups was 2.48 μ mol/mL Er, and the maximum 3.49 μ mol/mL Er. Analyzing the acquired values of the *t*-test, we found a statistically significant difference ($p < 0.001$) in the GSH values between the groups S and the control group. Analysis of the results of ANOVA-test proved a statistically significant difference in GSH values between the groups S : N, and S : M.

The median SOD values in the examined patients (groups S, M) and the control group (group N) were within the range of 1116.36–1313.23 IU/g Hgb (Table 4). The minimum value of SOD in the patients of all the three examined groups was 728 IU/g Hgb, and the maximum value was 1811 IU/g Hgb. Analyzing the acquired values of the *t*-test, we found a statistically significant difference ($p < 0.001$) in SOD values

Table 1
The distribution of the examined patients (groups S, M) and the control group (group N) according to the body weight (BW), body height (BH) and body mass index (BMI)

Characteristics of the patients	Group S (35)	Group M (35)	Group N (n = 50)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Age (years)	28.09 \pm 6.3	29.83 \pm 6.1	27.46 \pm 5.1
BM (kg)	61.23 \pm 11.9	65.77 \pm 10.7	62.55 \pm 11.1
BH (cm)	168.20 \pm 5.8	167.71 \pm 5.6	167.78 \pm 7.0
BMI (kg/m ²)	21.71 \pm 4.7	23.42 \pm 4.0	22.16 \pm 3.2

Group S – women with incomplete and complete spontaneous abortion;
Group M – women with missed abortion.

Table 2
The lipid peroxidation (LPx) values in the examined patients (groups S and M) and the control group (group N)

Groups	LPx (pmoL/mg Hgb)
	$\bar{x} \pm SD$
S (n = 35)	48.03 \pm 19.61*
M (n = 35)	44.57 \pm 16.48*
N (n = 50)	26.06 \pm 14.78

Hgb – hemoglobin;

* $p < 0.001$.

For explanation see under Table 1.

Table 3
Glutathione contents (GSH) in the examined patients (groups S, M) and the control group (group N)

Groups	GSH (μ mol/mL Er)
	$\bar{x} \pm SD$
S (n = 35)	2.68 \pm 0.16*
M (n = 35)	3.10 \pm 0.10
N (n = 50)	3.07 \pm 0.14

Er – erythrocytes;

* $p < 0.001$.

For explanation see under Table 1.

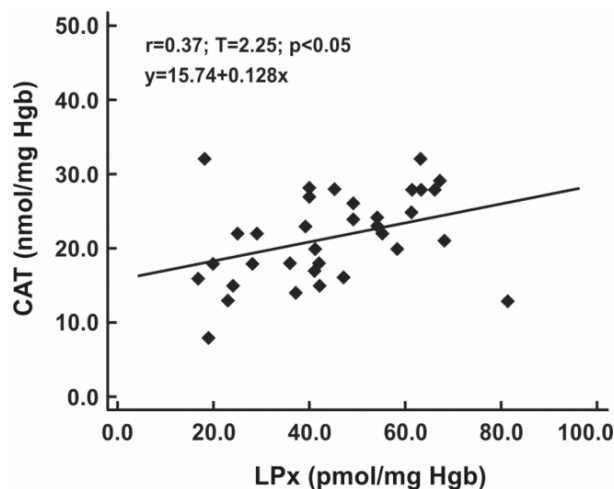
between the group S and the control group (N : S). The analysis of the ANOVA test proved the existence of a statistically significant difference in SOD values between the groups M : N; S : N, and S : M.

The median CAT values in the examined patients (S, M) and the control group (group N) were within the range of 20.40–30.94 nmoL/mg Hgb (Table 4). The minimum value of CAT in the patients of all the three examined groups was 8 nmoL/mg Hgb, and the maximum value was 96 nmoL/mg Hgb. Analyzing the acquired values of the *t*-test, we found a statistically significant difference ($p < 0.001$) in the CAT values between the examined patients and the control group (N : M; N : S). The analysis of the results of ANOVA test proved the existence of a statistically significant difference in the CAT values between the groups M : N, and S : N.

The median GSH-Px values in the examined patients (groups S, M) and the control group (group N) were within the range of 952.89–1291.38 nmoL/mg Hgb (Table 4). The minimum value of GSH-Px in the patients of all the three examined groups was 310 nmoL/mg Hgb, and the maximum value was 3,020 nmoL/mg Hgb. Analyzing the acquired values of the *t*-test, we found a statistically significant difference ($p < 0.05$) in the GSH-Px values between the examined patients and the control group (N : S). The analysis of the ANOVA test proved the existence of a statistically significant difference in GSH-Px values between the groups M : N; S : N.

The median TAS values in the examined patients (groups S, M) and the control group (group N) were within the range of 277.66–710.39 $\mu\text{mol/L}$ (Table 5). The minimum value of total antioxidative stress in the patients of all the three examined groups was 183.31 $\mu\text{mol/L}$, and the maximum value was 850.84 $\mu\text{mol/L}$. Analyzing the acquired values of the *t*-test, we found a statistically significant difference ($p < 0.001$) in the TAS values between the group S and the control group (N : M; N : S). The analysis of results of the ANOVA test proved the existence of a statistically significant difference in the TAS values between the groups M : N; S : N; and S : M.

There was a statistically positive correlation between LPx and CAT values ($p < 0.05$, $r = 0.37$) in the group M (Figure 1).



**Fig. 1 – Correlation between lipid peroxidation (LPx) (pmol/mg Hgb) and catalase (CAT) (nmol/mg Hgb) in the group M (women with missed abortion).
Hgb – hemoglobin.**

Discussion

The groups of pregnant women included in this study were homogenous in the age structure, body weight and height as well as in the gestation age of the pregnancy (Table 1). These parameters were not a risk factor in the occurrence of spontaneous abortions.

Lipid peroxidation is an oxidative damage the lipids, *ie* lipoproteins²⁰. Lipid peroxidation exists in the human population both in the early stages of pregnancy and in later gestation within the process of peroxidation^{21,22}. Elevated levels of lipid peroxidation as well as the degradation products can be found in the blood of pregnant women during normal pregnancy, especially in the state of preeclampsia. Analysis of LPx values

**Table 4
The values of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in the examined patients (groups S, M) and the control group (group N)**

Groups	SOD (IU/g Hgb)	CAT (nmol/mg Hgb)	GSH-Px (nmol/mg Hgb)
	$\bar{x} \pm \text{SD}$	$\bar{x} \pm \text{SD}$	$\bar{x} \pm \text{SD}$
S (n = 35)	1313.23 \pm 198.74***	20.40 \pm 9.06***	952.89 \pm 625.12*
M (n = 35)	1211.66 \pm 246.76	21.46 \pm 5.79***	1091.57 \pm 849.78
N (n = 50)	1116.36 \pm 175.96	30.94 \pm 17.71	1291.38 \pm 813.58

Hgb – hemoglobin; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

For explanation see under Table 1.

**Table 5
Total antioxidative status (TAS) values in the examined patients (groups S, M) and the control group (group N)**

Groups	TAS ($\mu\text{mol/L}$)
	$\bar{x} \pm \text{SD}$
S (n = 35)	710.39 \pm 78.34*
M (n = 35)	277.66 \pm 58.38*
N (n = 50)	452.12 \pm 74.40

* $p < 0.001$.

For explanation see under Table 1.

in our study showed the highest median values of LPx, found in the group of patients with spontaneous abortions (the group S – 48.03 pmol/mg Hgb), while the control group of healthy pregnancies had the lowest median values of LPx (the group N – 26.06 pmol/mg Hgb) (Table 2). A research conducted by Ozakaya et al.²³ as well as by Simšek et al.²⁴ yielded the same results. The increase of lipid peroxidation levels immediately before the onset of a spontaneous abortion and significantly lower values following the abortion were measured in the research by Sane et al.²⁵.

Our research showed a statistically important ($p < 0.05$, $r = 0.37$) positive correlation between lipid peroxidation and an antioxidative enzyme, catalase, in the group of patients with missed abortions (group M) (Figure 1). The increase in lipid peroxidation value was followed by the increase in catalase value.

SOD is a metalloenzyme which catalyzes the dismutation reaction of O_2^- to H_2O_2 with the change in the metal ion redox status (Cu^{2+} or Mn^{2+}) in the active center¹⁶. Mammals have two types of this enzyme: cytosolic homodimer CuZnSOD and mitochondrial homotetramer MnSOD. CAT "translates" high concentrations of H_2O_2 into the molecular oxygen and water quickly and can be found in peroxisomes.

Glutathione (γ -L-glutamyl-L-cysteinylglycine – GSH) is an α -amino acid as well as a tripeptide, which originates from glutamine, cysteine and glycine²⁶. Glutathione has a great number of functions such as metabolic, catalytic, transport and cell protection from oxidation and takes part in the transmembrane transport of amino acids, especially cysteine (and glutamine) which is necessary for protein synthesis^{27,28}. GSH-Px catalyzes the reduction of H_2O_2 and other organic hydroperoxides by using glutathione as a reductant, performing the function of protecting cell membrane lipids from oxidative stress²⁹. It has also been shown that selenium is an integral part of the enzyme glutathione peroxidase²⁹.

The highest median SOD value in our study was found in the group of patients with spontaneous abortions (the group S – 1,313.23 IU/g Hgb), while each of the two examined groups had equal median CAT values, and the control group of healthy pregnancies had the highest values (the group N – 30.94 nmoL/mg Hgb). The highest median GSH values in our study was found in the group of patients with missed abortions (the group M – 3.10 μ mol/ml Er), while the control group of patients had the highest median GSH-Px values (the group N – 1291.38 nmoL/mg Hgb) (Table 3).

Biri et al.³⁰ have examined the antioxidative enzyme values in the placental tissue of patients with normal pregnancies and patients with missed abortions. Their study showed significantly low SOD values, high CAT and GSH-Px values in the patients with missed abortions than in the control group. Some authors claim that there is a correlation between the occurrence of spontaneous abortions and low values of selenium in the plasma, as well as low GSH-Px activity. Similar results were found in a research by Ozkaya et al.²³, Prokopenko et al.³¹ and Sugino et al.³².

The Zachara et al.³³ examined the concentrations of Se-GSH and GSH-Px in the blood of patients with spontaneous abortions. The research included patients divided into three groups: the group I of 40 patients with the first trimester spontaneous abortions, the group II of 36 patients with the first trimester normal pregnancies, and the group III of 28 non-pregnant patients. Selenium concentrations in the full blood and plasma of the patients with spontaneous abortions and normal pregnancies were equal, while the selenium concentrations in non-pregnant patients were lower. Glutathione values were significantly higher in the patients with spontaneous abortions than in the patients with normal pregnancies and non-pregnant patients. GSH-Px values were significantly lower in the patients with spontaneous abortions than in the patients with normal pregnancies and non-pregnant patients. The same authors concluded that a decreased activity of antioxidative enzymes and GSH-Px may be a major etiological factor in spontaneous abortion occurrence.

The total antioxidative capacity represents the total capacity of the organism to protect itself from unwanted effects of physiological metabolism. The highest median TAS value in our study was found in the group of patients with spontaneous abortions (the group S – 710.39 μ mol/L) (Table 5), while the *t*-test values indicated a statistically highly significant difference in TAS values between the examined patients and the control group. Aksoy et al.³⁴ found a statistically significant decrease in total antioxidative status in patients with missed abortions in contrast to the patients with normal pregnancies. The decrease in total antioxidative status values in the patients with missed abortions may indicate that the damage of the total antioxidative system in the organism occurs³⁴. Panzan et al.³⁵ researched the antioxidative defense system in the patients with recurrent spontaneous abortions and found a decrease in nonenzymatic antioxidants in the plasma, as well as increased level of oxidative stress and damaged antioxidative defense system.

According to the literature data, the oxidative stress may influence the complete reproductive system of a woman, even in the period of menopause^{36–38}.

Conclusion

There was a statistically significant difference in the values of LPx and GSH as well as in the values of antioxidative enzymes activity: SOD, CAT and GSH-Px between the patients with spontaneous abortions and the control group.

The highest average value of the total antioxidative status (TAS) was recorded in the group of patients with spontaneous abortion. There was a statistically highly significant difference in the values of TAS between the examined patients and the control group.

Determining the values of pro-oxidative and antioxidative parameters in patients with spontaneous abortions may indicate the condition of the fetoplacental unit, and in the future these analyses could be included in the routine prenatal diagnostic protocol.

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