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Prostaglandin E_2, trace elements and levels of oxidative processes in spontaneous miscarriages

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Abstract. – OBJECTIVE: We investigated the significance of prostaglandin (PG)E_2, trace elements level, pro-oxidative and antioxidative parameters changes in spontaneous miscarriages.

PATIENTS AND METHODS: In the 125 first trimester pregnancies, 35 with complete spontaneous abortion (group S), 40 with missed abortion (M) and 50 healthy (H), PGE_2 plasma concentrations were analysed by commercial ELISA kits, plasma trace elements colorimetrically, lipid peroxidation and the antioxidative enzyme activities in hemolysate by commercial sets. Student's t-test and ANOVA were applied.

RESULTS: Average PGE_2 plasma concentration in the group H was higher than in S and M ($p < 0.05$). The higher average plasma Cu concentration, glutathione peroxidase and catalase contents were in the group H than in groups S and M ($p < 0.01$). Significantly lower value of lipid peroxidation was in the group H than in the groups S and M ($p < 0.01$). The lowest superoxide dismutase (SOD) content was in the group H and the highest in group S ($p < 0.01$).

CONCLUSIONS: In patients with abortions significantly lower levels of plasma PGE_2, plasma Cu and anti-oxidative enzymes, except SOD, and significantly higher level of lipid peroxidation products than in healthy pregnancies may be important for miscarriage etiology and prevention.

Key Words:
Spontaneous miscarriages, Prostaglandin E_2, Trace elements, Oxidative processes.

Introduction

Miscarriage prognosis, therapy and prevention may be better if we can control as many pregnancy parameters as we can, what implies subject of our research. We have investigated the significance of prostaglandin (PG)E_2, trace elements level, pro-oxidative and antioxidative parameters changes in spontaneous miscarriages.

It is demonstrated that prostaglandin (PG)E_2 release and production is required for mice embryo implantation[1]. Furthermore, the uterine epithelial Na^+ channel expression that leads to PGE_2 release, before *in vitro* fertilization treatment, is markedly lower in women with implantation failure as compared to those with successful pregnancy[1]. These indicate PGE_2 production and release role for human embryo implantation defects that may be a cause of miscarriage and low success rates of *in vitro* fertilization.

Significantly lower Cu concentrations were found in pathological conditions diagnosed in the first trimester of pregnancy, such as spontaneous abortus, threatened abortion, missed abortion, blighted ovum and intrauterine growth restriction, compared to normal pregnancy[2,3]. Opposite results were also reported[4]. Contradictory results were obtained with trace elements in pre-eclampsia[5,6].

Abnormal protein profiles associated with oxidative stress in decidual cells might be involved in the development of early pregnancy loss[7].

Increased lipid hydroperoxide plasma levels may play a role in miscarriage etiopathogenesis

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through increased susceptibility to lipid peroxidation (LPx) in women with early pregnancy failure[8].

A recent assessment[9] of oxidative stress markers in recurrent pregnancy loss supports the concept that oxidative stress plays a central role in the etiopathogenesis of spontaneously terminated pregnancy.

Our research provides additional parameters for pathophysiology and prevention of pregnancy loss.

The aim of this study was to find the possible significance of plasma PGE_2 level, plasma Cu and anti-oxidative enzymes, and level of lipid peroxidation products for miscarriage prevention and etiology explanations.

Patients and Methods

Study Design

This study was designed as a single-centre open-label, observational clinical trial for the investigation of the PGE_2 plasma concentrations, trace elements and levels of oxidative processes in spontaneously terminated pregnancies. The study protocol was in accordance with Declaration of Helsinki and was approved by the Ethic Committee of Medical Faculty, University of Novi Sad (Republic of Serbia) and the Clinical and Research Ethic Committee of Clinical Centre of Vojvodina, Novi Sad.

This longitudinal study was conducted from May 2013 to October 2014 in the Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Novi Sad.

Inclusion criteria for the study were: a) in the investigated - patient group - pregnant women with complete and with missed abortion in their first trimester of pregnancy, and b) in the control group - healthy pregnancies in the first trimester without any history of abortion. Exclusion criteria were: maternal medical problems, multiple pregnancies, systemic disease (e.g. hypertension and diabetes mellitus), pregnancy complications, alcohol, smoking or any drug use. All eligible subjects who provided written informed consent were entered in the study.

A total of 125 patients were involved in the research (75 spontaneous abortions and 50 healthy pregnancies). The patients were divided into groups: 35 patients with complete spontaneous abortion (group S), 40 patients with missed abortion (group M) and a control group of 50 healthy pregnancies (group H).

Samples

Blood samples (5 ml) were collected from the cubital vein, immediately after the establishment of a diagnosis of preterm delivery or missed abortion.

PGE_2 Determination

Blood samples were collected in sterile polypropylene tubes with EDTA. After centrifugation at the room temperature at 3000 g during 10 minutes, samples were immediately frozen and stored at -20ºC until the analysis.

Measurements of plasma levels of PGE_2 were performed by commercial ELISA kits (R&D Systems, Great Britain). Absorbance was measured in duplicates with a microplate reader (Beckman Coulter Inc., Brea, CA, USA). The final concentration was expressed in pg/ml. The sensitivity of the method was 41.4 pg/ml.

Determination of Trace Elements

Determination of trace elements in plasma was colorimetrical by the conventional methods for Cu[5,10], for Mg[11], for Ca[12] and Fe[13].

Oxidative Parameters Determination

The intensity of LPx was determined in hemolysate with the thiobarbituric acid method[14]. The antioxidative parameters in hemolysate were measured: catalase (Cat) with method by Aebi[15], the enzyme activity was measured by monitoring the decomposition of H_2O_2 at 240 nm, the activity of glutathione peroxidase (GPx) was determined using H_2O_2 as a substrate[16]. Superoxide dismutase (SOD) was measured by method of McCord and Fridovich[17] using xanthine oxidase.

Statistical Analysis

Experimental data were calculated as mean ± SE. Statistics were applied using Student's t-test for independent samples and ANOVA test to determine the level of significance, which was fixed at $p < 0.05$.

Results

Demographic characteristics of the patient and control groups are shown in Table I.

There was no statistically significant difference between demographic and clinical parameters of women in patient and control groups.

Table I. Demographic characteristics of investigated women.

Groups	Age years	Weight kg	Height cm	Gestat. age days	No of pregnancies	Parity frequency	
						1	1-4
Healthy N = 50	27.46 ± 5.17	62.55 ± 11.10	167.78 ± 7.01	79.82 ± 16.75	2	38	3
Spont. ab. N = 35	28.09 ± 6.34	61.23 ± 11.91	168.20 ± 5.83	63.66 ± 17.06	4	15	2
Miss. ab. N = 40	29.83 ± 6.17	65.77 ± 10.79	167.71 ± 5.66	58.06 ± 11.96	4	17	3

Also, patient and control groups were statistically compared regarding hemoglobin levels, activated partial thromboplastin time, platelet, red and white blood cells number and no significant difference was observed between the groups.

Average PGE_2 and trace elements plasma concentrations in healthy pregnancies, women with spontaneous and missed abortion are shown in Table II.

The hemolysate parameters of oxidative processes in healthy pregnancies, women with spontaneous and missed abortion are shown in Table III.

Average plasma PGE_2 concentration in the group H was the highest, compared to that in group S ($p < 0.05$) and M ($p < 0.05$). Also, a significant difference exists between groups S and M ($p < 0.01$).

Average plasma Cu concentration in the group H was the highest, compared to that in group M ($p < 0.01$) and group S ($p < 0.01$).

No statistically significant differences between groups were found for plasma levels of Mg, Ca, Fe and hemoglobin.

The lowest average value of LPx in hemolysate was recorded in the group H compared to that in group S ($p < 0.01$), and group M ($p < 0.01$).

The highest average GPx content in hemolysate was in the group H compared to that in group S ($p < 0.01$), and group M ($p < 0.01$).

The highest average hemolysate Cat content was also in the group H compared to that in group S ($p < 0.01$), and group M ($p < 0.01$).

The lowest average hemolysate SOD content was in the group H compared to that in group S ($p < 0.01$), and group M ($p > 0.05$, nonsignificant).

Discussion

In previous studies[1,18], there was no direct evidence of plasma PGE_2 changes in spontaneous abortions compared to the normal pregnancy.

Significantly lower plasma Cu concentrations in the case of spontaneous abortion and missed abortion, than in normal pregnancy in our study were in accordance with results of Alebic-Juretic

Table II. Prostaglandin E_2 and trace elements serum concentrations in healthy pregnancies, spontaneous and missed abortion groups

Groups	PGE_2 µg/l	Cu^{2+} µmol/l	Ca^{2+} mmol/l	Mg^{2+} nmol/l	Fe^{2+} µmol/l
Healthy N = 50	410.0 ± 139.8	28.43 ± 4.45	2.39 ± 0.34	0.88 ± 0.15	13.62 ± 12.46
Spont. ab. N = 35	285.0 ± 75.1	20.52 ± 3.76	2.38 ± 0.22	0.80 ± 0.14	14.60 ± 4.70
Miss. ab. N = 40	188.0 ± 57.3	21.40 ± 3.67	2.38 ± 0.19	0.89 ± 0.15	13.16 ± 4.28

Table III. The hemolysate components of oxidative processes in healthy pregnancies, spontaneous and missed abortion groups.

Groups	LPx pmol/mgHgb	GPx nmol/mgHgb	Cat nmol/mgHgb	SOD IU/gHgb
Healthy N 50	26.06 ± 14.78	1291.38 ± 813.58	30.94 ± 17.71	1116.36 ± 175.96
Spont. ab. N = 35	48.03 ± 19.61	952.89 ± 625.12	20.40 ± 9.06	1313.23 ± 198.74
Miss. ab. N = 40	44.57 ± 16.48	1091.57 ± 849.78	21.46 ± 5.79	1211.66 ± 246.76

LPx – lipid peroxidation; GPx – glutathione peroxidase; Cat – catalase; SOD – superoxide dismutase.

and Frkovic[2] and Shen et al[3] contrary to the results of Borella et al[4] who found higher Cu, lower Mg and not altered Ca concentrations in the case of spontaneous and threatened abortion.

Furthermore, Kumru et al[5] found that plasma Cu and Ca concentrations were significantly lower and Mg concentration unaltered in pre-eclamptic patients.

Farzin and Sajadi[6] found no difference in Cu and lower Ca and Mg levels in pre-eclampsia.

Simsek et al[19] found increased LPx and decreased antioxidant levels in patients with recurrent pregnancy loss, which is in accordance with our results.

Zachara et al[20] discovered that women with miscarriage had lowered GPx activity compared to viable pregnancies, which is also in agreement with our results.

El-Far et al[21] demonstrated that impairment of GPx, Cat, SOD antioxidant defense might be responsible for recurrent abortion. We can confirm such data, what could be useful for abortion risk determination.

In our study, the observed higher patients' SOD activity partly agree with the previously published results[22,23].

Many studies confirm that abnormal placentation in the first trimester leads to oxidative stress and resultant endothelial dysfunction plays a key role in the abortion, preeclampsia, and hydatidiform mole[24].

The exact pathophysiology of spontaneous abortion is still unknown, both cyclooxygenase-catalysed products and free radicals have been used as target of investigation of the pathophysiology diagnostics and prevention of early pregnancy complications[1,7-9,18-22,24].

Miscarriage prognosis, therapy and prevention may be better if we can control as many pregnancy parameters as we can, what implies possible subject of further research: determination of other PG levels in plasma, cervical mucus and saliva and other trace elements (Zn, Se) in plasma of patients with spontaneous miscarriages.

Our findings suggest that lower plasma levels of PGE_2, plasma Cu and hemolysate anti-oxidative enzymes GPx and Cat, and increased erythrocyte LPx may be used as additional parameters for diagnosis and prevention of pathological conditions in pregnancy, particularly in the first trimester. Furthermore, preterm delivery[25] and intrahepatic cholestasis of pregnancy[23] may be related to the PG concentrations and oxidative stress markers.

Conclusions

According to our results, significantly lower levels of plasma PGE_2, plasma Cu and hemolysate anti-oxidative enzymes, except SOD, and significantly increased erythrocyte LPx in pregnancy are accompanied by increased risk and even may be responsible for spontaneous and missed abortion. These, investigated level changes may be useful indicators for a miscarriage prevention.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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